

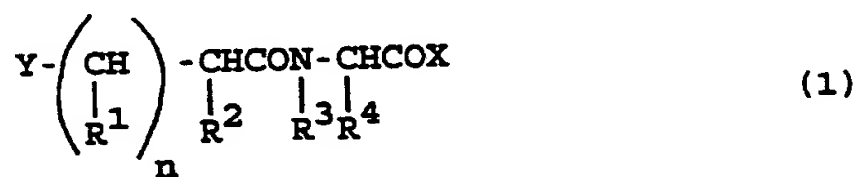
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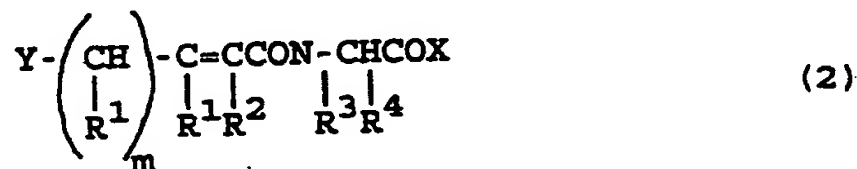
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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification⁵ : C07C 259/00, A61K 31/085 C07D 209/20, A61K 31/185 A61K 31/40	A1	(11) International Publication Number: WO 92/09563 (43) International Publication Date: 11 June 1992 (11.06.92)
(21) International Application Number: PCT/US91/08722 (22) International Filing Date: 21 November 1991 (21.11.91) (30) Priority data: 615,798 21 November 1990 (21.11.90) US 747,751 20 August 1991 (20.08.91) US (71) Applicant: GLYCOMED, INC. [US/US]; 860 Atlantic Avenue, Alameda, CA 94501 (US). (71)(72) Applicants and Inventors: GALARDY, Richard, E. [US/US]; 73 Faulkner Drive, Guilford, CT 06437 (US). GROBELNY, Damian [AU/AU]; 427 Canterbury Road, Surry Hills, VIC 3127 (AU). (72) Inventor: MUSSER, John, H. ; 10 Victoria Avenue, Macleod West, VIC 3085 (AU). (74) Agents: MURASHIGE, Kate, H. et al.; Morrison & Foerster, 545 Middlefield Road, Suite 200, Menlo Park, CA 94025 (US).		(81) Designated States: AT, AT (European patent), AU, BB, BE (European patent), BF (OAPI patent), BG, BJ (OAPI patent), BR, CA, CF (OAPI patent), CG (OAPI patent), CH, CH (European patent), CI (OAPI patent), CM (OAPI patent), CS, DE, DE (European patent), DK, DK (European patent), ES, ES (European patent), FI, FR (European patent), GA (OAPI patent), GB, GB (European patent), GN (OAPI patent), GR (European patent), HU, IT (European patent), JP, KP, KR, LK, LU, LU (European patent), MC, MG, ML (OAPI patent), MN, MR (OAPI patent), MW, NL, NL (European patent), NO, PL, RO, SD, SE, SE (European patent), SN (OAPI patent), SU*, TD (OAPI patent), TG (OAPI patent). Published <i>With international search report. Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>

(54) Title: IMPROVED MATRIX METALLOPROTEASE INHIBITORS

or

**(57) Abstract**

Compounds of formulas (1) or (2), wherein each R¹ is independently H or alkyl (1-8C) and R² is alkyl (1-8C) or wherein the proximal R¹ and R² taken together are -(CH₂)_p- wherein p = 3-5; R³ is H or alkyl (1-4C); R⁴ is fused or conjugated unsubstituted or substituted bicycloaryl methylene; n is 0, 1 or 2; m is 0 or 1; and X is OR⁵ or NHR⁵, wherein R⁵ is H or substituted or unsubstituted alkyl (1-12C), aryl (6-12C), aryl alkyl (6-16C); or X is an amino acid residue or amide thereof; r X is the residue of a cyclic amine or heterocyclic amine; Y is selected from the group consisting of R⁷ONR⁶CONR⁶-, R⁶₂NCONOR⁷-, and R⁶CONOR⁷-, wherein each R⁶ is independently H or lower alkyl (1-4C); R⁷ is lower alkyl (1-4C) or an acyl group; and wherein -CONR³- is optionally in modified isosteric form are inhibitors of matrix metalloproteases.

+ DESIGNATIONS OF "SU"

Any designation of "SU" has effect in the Russian Federation. It is not yet known whether any such designation has effect in other States of the former Soviet Union.

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IMPROVED MATRIX METALLOPROTEASE INHIBITORS

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Technical Field

The invention is directed to pharmaceuticals which are useful in diseases characterized by unwanted collagenase activity. More specifically, the invention concerns substituted or unsubstituted hydroxyureas and reverse hydroxamates and which include fused or conjugated bicycloaryl substituents.

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Background Art

There are a number of enzymes which effect the breakdown of structural proteins and which are structurally related metalloproteases. These include human skin fibroblast collagenase, human skin fibroblast gelatinase, human sputum collagenase and gelatinase, and human stromelysin. These are zinc-containing metalloprotease enzymes, as are the angiotensin-converting enzymes and the enkephalinases.

Collagenase and related enzymes are important in mediating the symptomology of a number of diseases, including rheumatoid arthritis (Mullins, D.E., et al., Biochim Biophys Acta (1983) 695:117-214); the metastasis of tumor cells (ibid., Broadhurst, M.J., et al., EP application 276436 (1987), Reich, R., et al., Cancer Res (1988) 48:3307-3312); and various ulcerated conditions.

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Ulcerative conditions can result in the cornea as the result of alkali burns or as a result of infection by Pseudomonas aeruginosa, Acanthamoeba, Herpes simplex and vaccinia viruses. Other conditions characterized by
5 unwanted matrix metalloprotease activity include periodontal disease, epidermolysis bullosa and scleritis.

In view of the involvement of collagenase in a number of disease conditions, attempts have been made to prepare inhibitors to this enzyme. A number of such
10 inhibitors are disclosed in EP applications 126,974 (published 1984) and 159,396 (published 1985) assigned to G.D. Searle. These inhibitors are secondary amines which contain oxo substituents at the 2-position in both substituents bonded to the amino nitrogen.

15 More closely related to the compounds of the present invention are those disclosed in U.S. patents 4,599,361 and 4,743,587, also assigned to G.D. Searle. These compounds are hydroxylamine dipeptide derivatives which contain, as a part of the compound, a tyrosine or
20 derivatized tyrosine residue or certain analogs thereof.

Other compounds that contain sulfhydryl moieties as well as residues of aromatic amino acids such as phenylalanine and tryptophan are disclosed in PCT application WO88/06890. Some of these compounds also
25 contain i-butyl side chains.

Inhibitors have also been disclosed for the related protease, thermolysin. These include hydroxamic peptide derivatives described by Nishino, N., et al., Biochemistry (1979) 18:4340-4347; Nishino, N., et al.,
30 Biochemistry (1978) 17:2846-2850. Tryptophan is also known to be therapeutic in various conditions, some of which may involve collagenase (see, for example, JP 57/058626; U.S. 4,698,342; 4,291,048). Also, inhibitors

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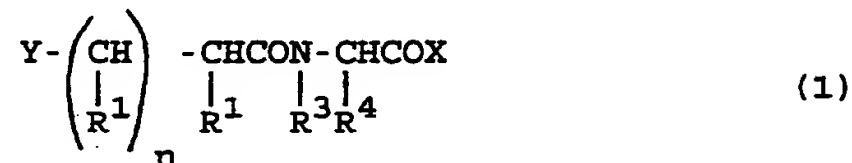
of bacterial collagenases have been disclosed in U.S.
4,558,034.

It has now been found that the compounds
described below have superior inhibiting activity with
5 respect to matrix metalloproteases. The invention
compounds add to the repertoire of agents available for
the treatment of conditions and diseases which are
characterized by unwanted activity by the class of
proteins which destroy structural proteins and designated
10 "matrix metalloprotease" herein.

Disclosure of the Invention

The invention provides new compounds which are
useful as inhibitors of matrix metalloproteases and which
15 are effective in treating conditions characterized by
excess activity of these enzymes. In addition, these
compounds can be used to purify matrix metalloproteases
and to detect increased levels of matrix metalloproteases
in vivo. The compounds take advantage of the incorpor-
20 ation of tryptophan or other fused or conjugated
bicycloaromatic amino acid residues into a substituted or
unsubstituted reverse hydroxamate- or a hydroxyurea-
derivatized matrix metalloprotease inhibitor.

Accordingly, in one aspect, the invention is
25 directed to compounds of the formula:

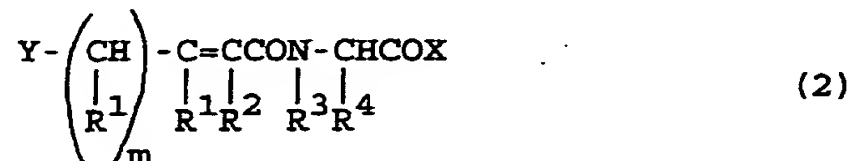


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or

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5 wherein each R^1 is independently H or alkyl (1-8C) and R^2 is alkyl (1-8C) or wherein the proximal R^1 and R^2 taken together are $-(\text{CH}_2)_p-$ wherein $p = 3-5$;

R^3 is H or alkyl (1-4C);

10 R^4 is fused or conjugated unsubstituted or substituted bicycloaryl methylene;

n is 0, 1 or 2;

m is 0 or 1; and

15 X is OR^5 or NHR^5 , wherein R^5 is H or substituted or unsubstituted alkyl (1-12C), aryl (6-12C), aryl alkyl (6-16C); or

X is an amino acid residue or amide thereof; or

X is the residue of a cyclic amine or heterocyclic amine;

20 Y is selected from the group consisting of $\text{R}^7\text{ONR}^6\text{CONR}^6-$, $\text{R}^6_2\text{NCONOR}^7-$, and $\text{R}^6\text{CONOR}^7-$, wherein each R^6 is independently H or lower alkyl (1-4C); R^7 is H, lower alkyl (1-4C) or an acyl group.

25 Also included within the scope of the invention are compounds wherein the $-\text{CONR}^3-$ amide bond shown is replaced by a modified isosteric bond, such as $-\text{CH}_2\text{NR}^3-$, $-\text{CH}_2\text{CHR}^3-$, $-\text{CH}=\text{CR}^3-$, $-\text{COCHR}^3-$, $-\text{CHOHCHR}^3-$, $-\text{NR}^3\text{CO}-$, $-\text{CF}=\text{CR}^3-$, and the like.

30 These compounds have the ability to inhibit at least one mammalian matrix metalloprotease. Accordingly, in other aspects, the invention is directed to pharmaceutical compositions containing the compounds of formula 1 or 2, to methods of treating diseases characterized by matrix metalloprotease activity using these compounds or the pharmaceutical compositions thereof. Matrix metallo-

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proteases at a particularly undesired location can be targeted by conjugating the compounds of the invention to a targeting ligand specific for a marker at that location such as an antibody or fragment thereof or a receptor ligand.

5 The invention is also directed to various other processes which take advantage of the unique properties of these compounds. Thus, in another aspect, the invention is directed to the compounds of formulas 1 or 2
10 conjugated to solid supports. These conjugates can be used as affinity reagents for the purification of a desired matrix metalloprotease.

In another aspect, the invention is directed to the compounds of formula 1 or 2 conjugated to label. As
15 the compounds of the invention selectively bind to at least one matrix metalloprotease, the label can be used to detect the presence of unusually large amounts of matrix metalloprotease in vivo or in vitro cell culture.

In addition, the compounds of formula 1 or 2
20 can be conjugated to carriers which permit the use of these compounds in immunization protocols to prepare antibodies specifically immunoreactive with the compounds of the invention. These antibodies are then useful both in therapy and in monitoring the dosage of the
25 inhibitors.

In still another aspect, the invention is directed to methods to prepare the compounds of formulas 1 and 2 and to novel intermediates in their preparation.

30 Brief Description of the Drawings

Figure 1 is a graph which shows the effect of the inhibitor of the invention on corneal burns using a clinical scoring method.

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Figure 2 shows the corresponding effect of the compound of the invention on corneal burns using a perforation criterion.

5 Modes of Carrying Out the Invention

The invention compounds are inhibitors of mammalian matrix metalloproteases. As used herein, "mammalian matrix metalloprotease" means any enzyme found in mammalian sources which is capable of catalyzing the breakdown of collagen, gelatin or proteoglycan under suitable assay conditions. Appropriate assay conditions can be found, for example, in U.S. patent 4,743,587, which references the procedure of Cawston, et al., Anal Biochem (1979) 99:340-345, use of a synthetic substrate is described by Weingarten, H., et al., Biochem Biophys Res Comm (1984) 139:1184-1187. Any standard method for analyzing the breakdown of these structural proteins can, of course, be used. The matrix metalloprotease enzymes referred to in the herein invention are all zinc-containing proteases which are similar in structure to, for example, human stromelysin or skin fibroblast collagenase.

The ability of candidate compounds to inhibit matrix metalloprotease activity can, of course, be tested in the assays described above. Isolated matrix metalloprotease enzymes can be used to confirm the inhibiting activity of the invention compounds, or crude extracts which contain the range of enzymes capable of tissue breakdown can be used.

30 The invention compounds can be considered to comprise two components linked by an amide or modified amide bond. One component is substituted or unsubstituted hydroxyurea or reverse hydroxamate derivative of a dicarboxylic acid backbone, wherein the

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alternate carboxyl group forms the amide or modified bond with an amino acid or analog thereof. The amino acid or analog is the residue of an amino acid or analog which contains a fused or conjugated bicycloaromatic system, such as a tryptophan residue or a naphthylalanyl residue. This residue can also be amidated or can be extended by one or two additional amino acid residues. Thus, the compounds of the invention can be prepared by the methods described below.

10 The dicarboxylic acid residue in formula 1 contains at least one and, in some instances, two or more chiral centers. Either configuration at any chiral center is included within the invention, as are mixtures of compounds containing the two possible configurations at each point of chirality. However, it is generally found that a particular configuration at each of these chiral centers is preferred. Similarly, in the compounds of formula 2, the double bond can be either the cis or trans configuration. In this case, also, one or the other configuration for a particular set of embodiments will be preferred. The carbon to which R^4 is bound is chiral in both formulas. While both configurations are included in the invention, that corresponding to an L-amino acid is preferred.

25 The amide bond shown as $-\text{CONR}^3-$ in the compounds of formulas 1 and 2 may be in "modified isosteric" form. Compounds of the invention where this is the case are preferred when oral administration is desirable. By "modified isosteric form" is meant that in lieu of the functionality $-\text{CONR}^3-$, the compound has instead a moiety such as those selected from the group consisting of $-\text{CH}_2\text{NR}^3-$, $-\text{CH}-\text{NR}^3$, $-\text{COCHR}^3-$, $-\text{CH}(\text{OH})\text{NR}^3$, $-\text{CH}(\text{OH})\text{CHR}^3-$, $-\text{CSCHR}^3-$, $-\text{CH}=\text{CR}^3-$, $\text{CF}=\text{CR}^3$ and $-\text{NR}^3\text{CO}-$.

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As used herein, "alkyl" has its conventional meaning as a straight chain, branched chain or cyclic saturated hydrocarbyl residue such as methyl, ethyl, isobutyl, cyclohexyl, t-butyl or the like. The alkyl substituents of the invention are of the number of carbons noted which may be substituted with 1 or 2 substituents. Substituents are generally those which do not interfere with the activity of the compound, including hydroxyl, "CBZ," amino, and the like. Aryl refers to aromatic ring systems such as phenyl, naphthyl, pyridyl, quinolyl, indolyl, and the like; aryl alkyl refers to aryl residues linked to the position indicated through an alkyl residue. In all cases the aryl portion may be substituted or unsubstituted. "Acyl" refers to a substituent of the formula RCO- wherein R is alkyl or arylalkyl as above-defined. The number of carbons in the acyl group is generally 1-15; however, as the acyl substituent is readily hydrolyzed in vivo, the nature of the group is relatively unimportant. "Cyclic amines" refer to those amines where the nitrogen is part of a heterocyclic ring, such as piperidine, "heterocyclic amines" refer to such heterocycles which contain an additional heteroatom, such as morpholine.

In the compounds of formula 1, preferred embodiments for R^1 and R^2 include those wherein each R^1 is H or Me and R^2 is alkyl of 3-8C, especially isobutyl, 2-methyl butyl, or isopropyl. Especially preferred is isobutyl. Preferred also are those compounds of formula 1 or 2 wherein $n=1$ or $m=1$.

In both formula 1 and 2 compounds, preferred embodiments of R^3 are H and methyl, especially H.

R^4 is a fused or conjugated bicyclo aromatic system linked through a methylene group to the molecule. By "fused or conjugated bicyclo aromatic system" is meant

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a two-ringed system with aromatic character which may, further, contain one or more heteroatoms such as S, N, or O. When a heteroatom such as N is included, the system as it forms a part of formula (1) or (2), may contain an acyl protecting group (1-5C) attached to the nitrogen. Representative bicyclo fused aromatic systems include naphthyl, indolyl, quinolinyl, and isoquinolinyl. Representative conjugated systems include biphenyl, 4-phenylpyrimidyl, 3-phenylpyridyl and the like. In all cases, any available position of the fused or conjugated bicyclic system can be used for attachment through the methylene. The fused or conjugated aromatic system may further be substituted by 1-2 alkyl (1-4C) residues and/or hydroxy or any ring nitrogens may be acylated. Preferred acylation is acetylation.

Preferred embodiments of R^4 include 1-(2-methylnaphthyl)methylene; 1-quinolyl methylene; 1-naphthyl methylene; 2-naphthyl methylene; 1-isoquinolyl methylene; 3-isoquinolyl methylene; 3-thionaphthenyl methylene; 3-cumaronyl methylene; 3-(5-methylindolyl)methylene; 3-(5-hydroxyindolyl)methylene; 3-(2-hydroxyindolyl)-methylene; biphenyl methylene; and 4-phenylpyrimidyl methylene; and the substituted forms thereof.

Many of these substituents as part of an amino acid residue are described in Greenstein and Winitz, "Chemistry of the Amino Acids" (1961) 3:2731-2741 (John Wiley & Sons, NY).

A particularly preferred embodiment of R^4 is 3-indolyl methylene or its N-acylated derivative--i.e., that embodiment wherein the "C-terminal" amino acid is a tryptophan residue or a protected form thereof. A preferred configuration at the carbon to which R^4 is bound is that corresponding to L-tryptophan.

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Preferred embodiments of X are those of the formula NHR^5 wherein R^5 is H, substituted or unsubstituted alkyl (1-12C) or aryl alkyl (6-12C). Particularly preferred substitutions on R^5 are a hydroxyl group, or a phenylmethoxycarbonyl (CBZ) residue. In addition, the compound may be extended by embodiments wherein X is an additional amino acid residue, particularly a glycyl residue, which may also be amidated as described.

10 Therapeutic Use of the Compounds of the Invention

As set forth in the Background section above, a number of diseases are known to be mediated by excess or undesired matrix-destroying metalloprotease activity. These include tumor metastasis, rheumatoid arthritis, skin inflammation, ulcerations, particularly of the cornea, reaction to infection, and the like. Thus, the compounds of the invention are useful in therapy with regard to conditions involving this unwanted activity.

The invention compounds can therefore be formulated into pharmaceutical compositions for use in treatment or prophylaxis of these conditions. Standard pharmaceutical formulation techniques are used, such as those disclosed in Remington's Pharmaceutical Sciences, Mack Publishing Company, Easton, PA, latest edition.

For indications to be treated systemically, it is preferred that the compounds be injected. These conditions include rheumatoid arthritis and tumor metastasis. The compounds can be formulated for injection using excipients conventional for such purpose such as physiological saline, Hank's solution, Ringer's solution, and the like. Injection can be intravenous, intramuscular, intraperitoneal or subcutaneous. Dosage levels are of the order of 0.1 $\mu\text{g/kg}$ of subject to 1 mg/kg of subject, depending, of course, on the nature

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of the condition, the nature of the subject, the particular embodiment of the invention compounds chosen, and the nature of the formulation and route of administration.

In addition to administration by injection, the compounds of the invention can also be formulated into compositions for transdermal or transmucosal delivery by including agents which effect penetration of these tissues, such as bile salts, fusidic acid derivatives, cholic acid, and the like. The invention compounds can also be used in liposome-based delivery systems and in formulations for topical and oral administration depending on the nature of the condition to be treated. Oral administration is especially advantageous for those compounds wherein the moiety $-\text{CONR}^3-$ is in a modified isosteric form. These compounds resist the hydrolytic action of the digestive tract. Oral formulations include syrups, tablets, capsules, and the like, or the compound may be administered in food or juice.

The inhibitors of the invention can be targeted to specific locations where the matrix metalloprotease is accumulated by using targeting ligands. For example, to focus the inhibitors to matrix metalloprotease contained in a tumor, the inhibitor is conjugated to an antibody or fragment thereof which is immunoreactive with a tumor marker as is generally understood in the preparation of immunotoxins in general. The targeting ligand can also be a ligand suitable for a receptor which is present on the tumor. Any targeting ligand which specifically reacts with a marker for the intended target tissue can be used. Methods for coupling the invention compound to the targeting ligand are well known and are similar to those described below for coupling to carrier. The conjugates are formulated and administered as described above.

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For localized conditions, topical administration is preferred. For example, to treat ulcerated cornea, direct application to the affected eye may employ a formulation as eyedrops or aerosol. For corneal treatment, the compounds of the invention can also be formulated as gels or ointments, or can be incorporated into collagen or a hydrophilic polymer shield. The materials can also be inserted as a contact lens or reservoir or as a subconjunctival formulation. For treatment of skin inflammation, the compound is applied locally and topically, in a gel, paste, salve or ointment. The mode of treatment thus reflects the nature of the condition and suitable formulations for any selected route are available in the art.

Particular topical conditions that are susceptible to treatment using the compounds of the invention include stomach ulcers, superficial wounds of any type, epidermolysis bullosa, various forms of skin cancer, and pemphigus. It is well known that collagenase is involved in the progress of stomach ulcers, as described by Hasabe, T., et al., J Exp Clin Med (1987) 12:181-190; Ozaki, I., et al., Scand J Gastroenterol Suppl (Norway) (1989) 16:138-141. Collagenase is also found in marginal wound tissue as reviewed by Mullins, D.E., et al., Biochim Biophys Acta (1983) 695:177-214, and patients showing impaired wound healing are known to have increased collagenase activity in the wound tissue. For example, Hennessey, P.J., et al., J Pediat Surg (1990) 25:75-78, showed this to be the case in diabetes; Sank, A., et al., Surgery (1989) 106:1141-1148, demonstrated this enhanced collagenase activity in paraplegia, chronic renal failure, and Cushings disease. Thus, the collagenase inhibitors of the invention are useful in any ulcerative skin condition, including, for example,

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decubitus ulcers, or other conditions where wound healing is slow; other conditions susceptible to treatment by the compounds of the invention include corneal or scleral melting associated with keratomalacia, scleromalacia perforans and connective tissue diseases.

5 This is also the case with respect to wounds inflicted in the course of medical treatment. It has been demonstrated that collagenase inhibitors enhance the strength of suture lines and suture holding capacity in intestinal anastomoses in rats (Hogstrom, H., et al., Res
10 Exp Med (1985) 185:451-455. Other topical conditions responsive to the collagenase inhibitors of the invention include epidermolysis bullosa where increased collagenase activity has been demonstrated (Perez-Tamayo, R., Am J
15 Path (1978) 92:509-566, pp. 539-541). It is also known that collagenase activity is increased in the stroma surrounding basal cell carcinoma and throughout tumor stromas in ulcerated basal cell carcinoma (Childer, S., J.W., et al., J Am Acad Dermatol (1987) 17:1025-1032).

20 Further, the matrix metalloprotease inhibitors of the invention are useful in the treatment of septic shock and in the treatment of adult respiratory distress syndrome (ARDS). The role of plasma proteases in septic shock has been shown by Colman, R.C., New Eng J Med
25 (1989) 320:1207-1210, and the participation of neutrophil proteases in both septic shock and ARDS has been shown by Idell, S., et al., Ann Res Respir Dis (1985) 132:1098-1105. In the case of these conditions, the compounds of the invention protect the connective tissue directly from
30 digestion by matrix metalloproteases as well as prevent the degradation of collagen and therefore prevention of known chemotaxis of neutrophils toward collagen fragments (Werb, Z., in "Textbook of Rheumatology," Kelley, W.N., et al., eds. (1989) W.B. Saunders, Philadelphia, 3rd ed.,
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pp. 300-320). The compounds of the invention also prevent the inactivation of plasma protease inhibitors by matrix metalloproteases (Werb, Z. supra).

5 In all of the foregoing, of course, the compounds of the invention can be administered alone or as mixtures, and the compositions may further include additional drugs or excipients as appropriate for the indication.

10 Some of the compounds of the invention also inhibit bacterial metalloproteases although generally at a lower level than that exhibited with respect to mammalian metalloproteases. Some bacterial metalloproteases seem to be less dependent on the stereochemistry of the inhibitor, whereas substantial
15 differences are found between diastereomers in their ability to inactivate the mammalian proteases. Thus, this pattern of activity can be used to distinguish between the mammalian and bacterial enzymes.

20 Preparation and Use of Antibodies

The invention compounds can also be utilized in immunization protocols to obtain antisera immunospecific for the invention compounds. As the invention compounds are relatively small haptens, they are advantageously
25 coupled to antigenically neutral carriers such as the conventionally used keyhole limpet hemocyanin (KLH) or serum albumin carriers. Coupling to carrier can be done by methods generally known in the art; the -COX functionality of the invention compounds offers a
30 particularly convenient site for application of these techniques. For example, the COX residue can be reduced to an aldehyde and coupled to carrier through reaction with sidechain amino groups in protein-based carriers, optionally followed by reduction of imino linkage formed.
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The COX residue wherein X=OH can also be reacted with sidechain amino groups using condensing agents such as dicyclohexyl carbodiimide or other carbodiimide dehydrating agents. Linker compounds can also be used to effect the coupling; both homobifunctional and heterobifunctional linkers are available from Pierce Chemical Company, Rockford, IL. Compounds 31-34 described in the examples below are designed to be coupled to antigenically neutral carriers through their C-terminal carboxyl groups (or amino groups in compound 32) using appropriate coupling agents.

The resulting immunogenic complex can then be injected into suitable mammalian subjects such as mice, rabbits, and the like. Suitable protocols involve repeated injection of the immunogen in the presence of adjuvants according to a schedule which boosts production of antibodies in the serum. The titers of the immune serum can readily be measured using immunoassay procedures, now standard in the art, employing the invention compounds as antigens.

The antisera obtained can be used directly or monoclonal antibodies may be obtained by harvesting the peripheral blood lymphocytes or the spleen of the immunized animal and immortalizing the antibody-producing cells, followed by identifying the suitable antibody producers using standard immunoassay techniques.

The polyclonal or monoclonal preparations are then useful in monitoring therapy or prophylaxis regimens involving the compounds of the invention. Suitable samples such as those derived from blood, serum, urine, or saliva can be tested for the presence of the administered inhibitor at various times during the treatment protocol using standard immunoassay techniques which employ the antibody preparations of the invention.

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The invention compounds can also be coupled to labels such as scintigraphic labels, e.g., technetium 99 or I-131, using standard coupling methods. The labeled compounds are administered to subjects to determine the locations of excess amounts of one or more matrix metalloproteases in vivo. The ability of the inhibitors to selectively bind matrix metalloprotease is thus taken advantage of to map the distribution of these enzymes in situ. The techniques can also, of course, be employed in histological procedures and the labeled invention compounds can be used in competitive immunoassays.

Use As Affinity Ligands

The invention compounds can be coupled to solid supports, such as separation membranes, chromatographic supports such as agarose, sepharose, polyacrylamide, and the like, or to microtiter plates to obtain affinity supports useful in purification of various mammalian matrix metalloproteases. The selective binding of the matrix metalloproteases to the inhibitor ligand permits the adsorption of the desired enzyme and its subsequent elution using, for example, altered ionic strength and/or pH conditions.

Preparation of the Invention Compounds

The reverse hydroxamates and hydroxyureas are more stable biologically than the corresponding hydroxamates per se. This has been confirmed in Carter, G.W., et al., J Pharmacol Exp Ther (1991) 256:929-937; Jackson, W.P., et al., J Med Chem (1988) 31:499-500; Young, P.R., et al., FASEB J (1991) 5:A1273; Hahn, R.A., et al., J Pharmacol Ex Ther (1991) 256:94-102; Tramposch, K.M., et al., Agents Actions (1990) 30:443-450; Argentieri, D.C., et al.; Kimball, E., et al., 5th Int

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Conf Inflammation Research Assoc., Whitehaven, PA, September 23-27, 1990, Abstract 100; and Huang, F., et al., J Med Chem (1989) 32:1836-1842. Thus, while somewhat more complicated to synthesize, these analogs offer physiological characteristics which are advantageous in the applications of these compounds to therapy.

The reverse hydroxamates and hydroxyureas of the invention are obtainable using the standard techniques of synthetic organic chemistry (see Challis, B.C., et al., "Amides and Related Compounds" in "Comprehensive Organic Chemistry," Barton, D., et al., eds. (1979) 2:1036-1045), Pergamon Press, Oxford, as further described below.

With respect to starting materials, the components forming the $-NR^3-CHR^4COX$ moiety are readily available in the case of tryptophan and its analogs as esters or amides. As set forth above, many analogous fused bicyclo aromatic amino acids are described by Greenstein and Winitz (supra). Amino acids corresponding to those wherein R^4 is 1-(2-methyl naphthyl)methylene; 1-quinolyl-methylene; 1-naphthyl methylene; 1-isoquinolyl methylene; and 3-isoquinolyl methylene can be prepared from the bicyclo aromatic methylene halides using the acetamido malonic ester synthesis of amino acids, as is well understood in the art. The methylene halides themselves can be prepared from their corresponding carboxylic acids by reduction with lithium aluminum hydride and bromination of the resulting alcohol with thionyl bromide.

Depending on the functional group symbolized by Y, the stage of synthesis at which this moiety is brought into the compound of the invention varies.

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For those embodiments wherein Y is $R^7ONR^6CONR^6-$ and wherein $n = 0, 1$ or 2 , the compounds are prepared by acylating an α , β or γ amino acid, respectively with methyl or ethyl chloroformate, condensing the resulting amino acid with a protected form of the moiety
5 $-NR^3CHR^4COX$ and reacting the resulting carboethoxy "dipeptide" with hydroxylamine or a substituted hydroxylamine as described by Fieser, L.F., et al., "Reagents for Organic Synthesis" (1967) 1:479 (John Wiley
10 & Sons, New York). This sequence of reactions is shown in Reaction Scheme 1.

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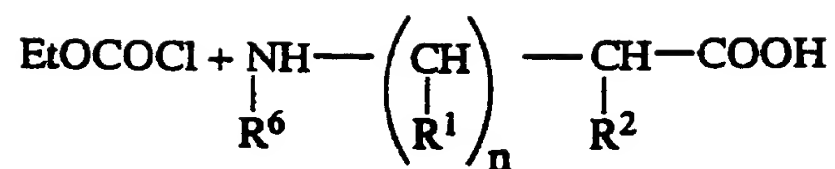
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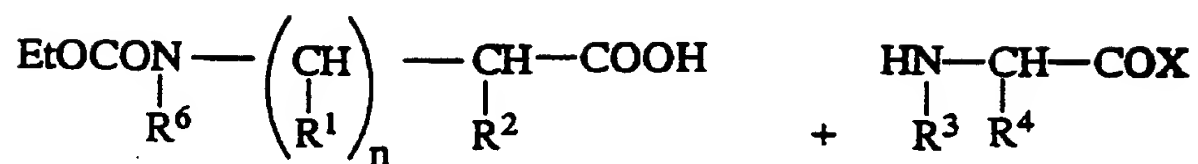
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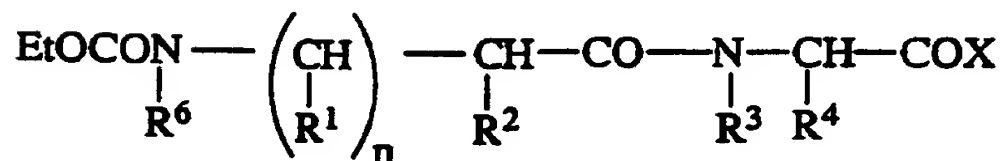
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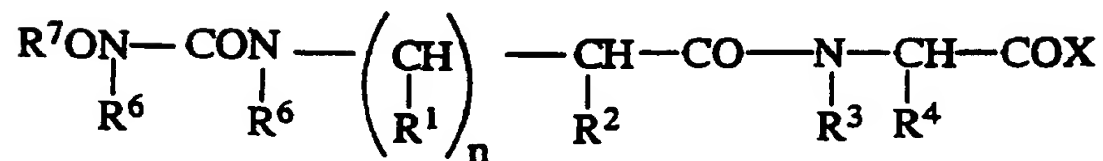
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REACTION SCHEME 1

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Alternatively, the α , β or γ amino acid is temporarily protected using, for example, carbobenzoxy or tertiary butyloxycarbonyl and coupling it to the carboxy-terminal-protected amino acid moiety containing R^4 . The
5 protecting group is then removed by hydrogenolysis or acidolysis as appropriate, and the deprotected α , β or γ amino group is reacted with an activated carbonic acid such as carbonyldiimidazole. The resultant is then
10 reacted with hydroxylamine or substituted hydroxylamine to obtain the desired product. This sequence of reactions is summarized in Reaction Scheme 2. (In the formula Im-Co-Im, Im represents an imidazole residue.)

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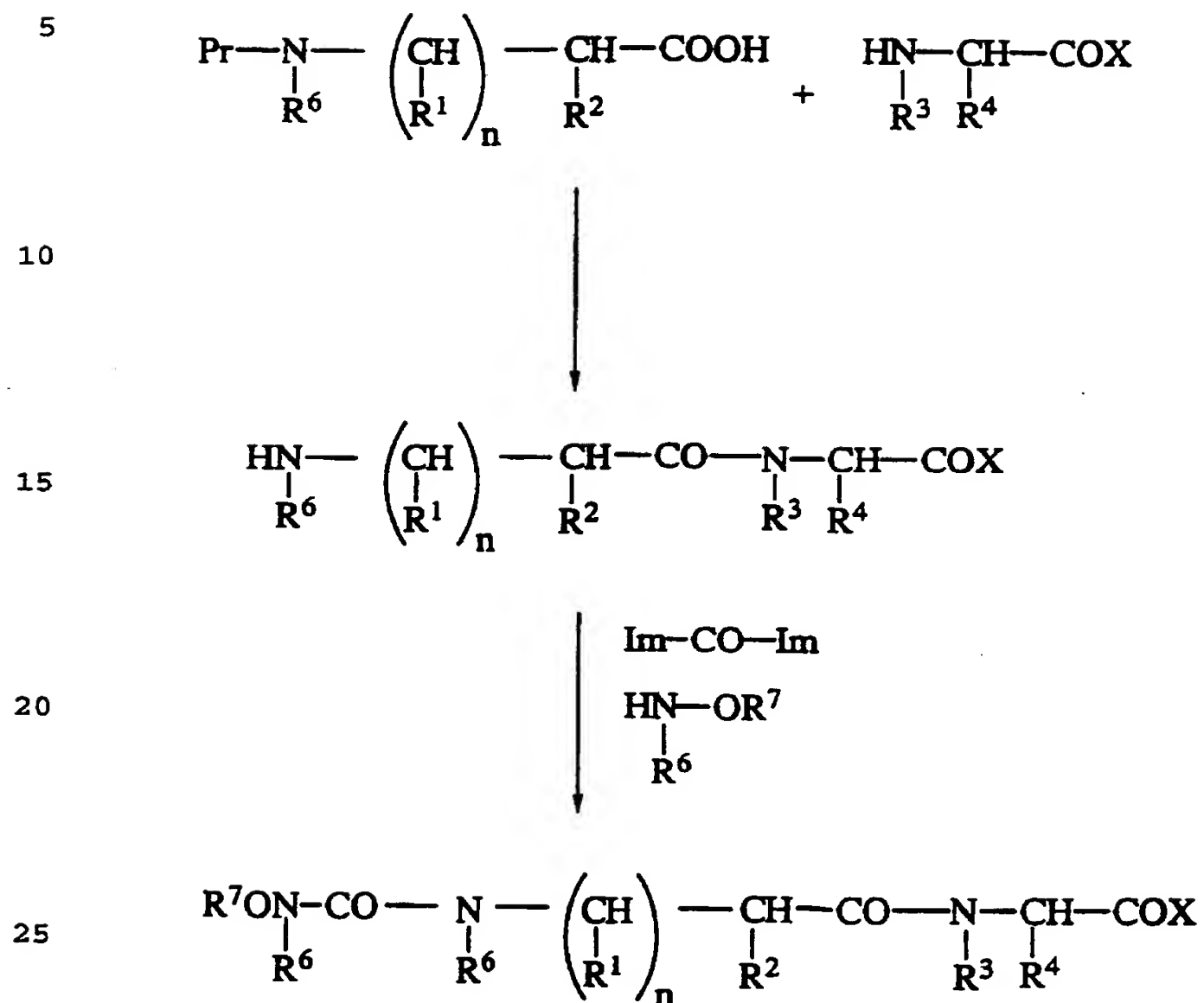
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REACTION SCHEME 2

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The appropriate α , β or γ amino acids are prepared by general methods as set forth by Jones, J.H., et al., in "Amino Acids," p. 834 (Barton, D., et al., eds.) ("Comprehensive Organic Chemistry" (1979) Vol. 2, Pergamon Press). Such methods include, for example, homologation by Arndt-Eistert synthesis of the corresponding N-protected α -amino acid and more generally the addition of nitrogen nucleophiles such as phthalimide to α, β -unsaturated esters, acids or nitriles.

10 In a second class of hydroxyureas, Y has the formula $R^6_2NCONOR^7$ and n is 0, 1 or 2. These compounds are prepared from the corresponding α , β or γ hydroxyamino acids of the formula $R^7ONH(CHR^1)_nCHR^2COOH$. When both R^6 are H, this intermediate is converted to the
15 desired hydroxyurea by reaction with silicon tetraisocyanate, as described by Fieser and Fieser, "Reagents for Organic Synthesis" (1968) 1:479 (John Wiley & Sons, New York). The reaction is conducted with the hydroxyl group protected or substituted by R^7 . The
20 resulting hydroxyurea is then coupled to the component of the formula HNR^3CHR^4COX to obtain the desired product. Alternatively, the amide is first formed and the N-hydroxyl dipeptide is treated with the reagent.

Alternatively, when Y is $R^6HNCO-NOR^7$, wherein
25 R^6 is alkyl, the above O-protected α , β or γ N-hydroxyamino acid is reacted with the relevant alkylisocyanate R^6NCO to produce the desired product.

When Y is of the formula $R^6_2NCO-NOR^7$ wherein both R^6 are alkyl, the α , β or γ N-hydroxyamino acid is
30 reacted with an activated form of carbonic acid, for example, carbonyldiimidazole or bis-p-nitrophenylcarbonate, and then with the diamine R^6_2NH wherein both R^6 are alkyl groups. This is followed by deprotection, if
35 desired.

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Conditions for the foregoing can be found in the descriptions of analogous preparations for tripeptides as described by Nishino, N., et al., Biochemistry (1979) 18:4340-4346.

5 The β -N-hydroxyamino acids used as intermediates in the foregoing synthesis can be prepared by a malonic ester synthesis in which diethyl malonate is alkylated twice, one with R^2 -Br and then with benzylchloromethyl ether, for example, for the case
10 wherein R^1 is H. The product is saponified, decarboxylated, hydrogenated, and oxidized to give the β -aldehyde in a manner similar to the synthesis of a homologous aldehyde described by Kortylewicz, Z.P., et al., Biochemistry (1984) 23:2083-2087. The desired β -
15 hydroxyamino acid is then obtained by addition of protected (or alkylated, if R^7 is alkyl or acylated if R^7 is acyl) hydroxylamine. The corresponding compound wherein R^1 is alkyl can be prepared in an analogous manner wherein the second alkylation utilizes benzyl-O-
20 CHR^1Cl . The homologous ketone was described by Galardy, R.E., et al., Biochemistry (1985) 24:7607-7612.

Finally, those compounds wherein Y is of the formula R^6CONOR^7 -, i.e., the reverse hydroxymates, can be prepared by acylation of the corresponding α , β or γ N-
25 hydroxy dipeptide. Alternatively, the N-hydroxyamino acid can be acylated, followed by condensation to form the amide bond in the compounds of the invention. The acylation method is described by, for example, Nishino, N., et al., Biochemistry (1979) 18:4340-4346, cited
30 above.

Alternatively, for those compounds wherein $n=1$ and R^1 is H, the compounds can be prepared by condensing the ylide 1,1-dimethoxy-2-(triphenylphosphoranylidene) ethane prepared from triphenylphosphine and 1,1-
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dimethoxy-2-bromoethane with 4-methyl-2-oxopentanoic acid. The product is then hydrogenated to obtain 4,4-dimethoxy-2-isobutylbutanoic acid which is coupled to the moiety R^3NHCHR^4COX to obtain 4,4-dimethoxy-2-isobutylbutanoyl- NR^3CHR^4COX . Treatment with aqueous acid yields the aldehyde 2-isobutyl-4-oxobutanoyl- NR^3CHR^4COX . The oxime is prepared by reaction with hydroxylamine and reduced to the corresponding N-substituted hydroxylamine. Acylation of both the hydroxaminol oxygen and nitrogen followed by hydrolysis of the O-acyl group provides the N-acyl reverse hydroxymates. (Summers, J.B., et al., J Med Chem (1988) 31:1960-1964.)

For compounds wherein $-CONR^3-$ is in modified isosteric form, these forms can be prepared by methods known in the art. The following references describe preparation of peptide analogs which include these alternative-linking moieties: Spatola, A.F., Vega Data (March 1983), Vol. 1, Issue 3, "Peptide Backbone Modifications" (general review); Spatola, A.F., in "Chemistry and Biochemistry of Amino Acids Peptides and Proteins," B. Weinstein, eds., Marcel Dekker, New York, p. 267 (1983) (general review); Morley, J.S., Trends Pharm Sci (1980) pp. 463-468 (general review); Hudson, D., et al., Int J Pept Prot Res (1979) 14:177-185 ($-CH_2NR^3-$, $-CH_2CHR^3-$); Spatola, A.F., et al., Life Sci (1986) 38:1243-1249 ($-CH_2-S-$); Hann, M.M., J Chem Soc Perkin Trans I (1982) 307-314 ($-CH-CR^3-$, cis and trans); Almquist, R.G., et al., J Med Chem (1980) 23:1392-1398 ($-COCHR^3-$); Jennings-White, C., et al., Tetrahedron Lett (1982) 23:2533 ($-COCHR^3-$); Szelke, M., et al., European Application EP 45665 (1982) CA:97:39405 (1982) ($-CH(OH)CHR^3-$); Holladay, M.W., et al., Tetrahedron Lett (1983) 24:4401-4404 ($-C(OH)CH_2-$); and Hruby, V.J., Life Sci (1982) 31:189-199 ($-CH_2-S-$).

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The following examples are intended to illustrate but not to limit the invention.

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EXAMPLES

In the examples below, TLC solvent systems are as follows: (A) ethyl acetate/methanol (95:5); (B) ethyl acetate/methanol (25:5); (C) ethyl acetate; (D) ethyl acetate/methanol (30:5); (E) ethyl acetate/hexane (1:1);
10 (F) chloroform/methanol/acetic acid (30:6:2); (G) chloroform/methanol/acetic acid (85:10:1).

Example 1

Preparation of N-[D,L-2-isobutyl-3-(N'-
15 hydroxycarbonylamido)-propanoyl]-tryptophan methylamide

A suspension of 5 g (0.033 mol) of the sodium salt of 4-methyl-2-oxopentanoic acid and 5.65 g (0.033 mol) of benzyl bromide in 10 ml of anhydrous dimethylformamide was stirred for 4 days at room
20 temperature. After evaporation of the solvent under reduced pressure the residue was diluted to 100 ml with hexane and washed with water (3 x 20 ml) and saturated sodium chloride and dried over anhydrous magnesium sulfate. Evaporation of solvent gave 6.4 g (88% yield)
25 of the benzyl ester of 4-methyl-2-oxopentanoic acid (1) as a colorless oil.

A mixture of 6.4 g (0.029 mol) of (1) and 9.7 g (0.029 mol) of methyl(triphenylphosphoranylidene)acetate in 100 mL of dry methylene chloride was stirred for 12 hr
30 at room temperature and evaporated to dryness. The residue was extracted with hexane (3 x 50 mL). The hexane solution was washed with 10% sodium bicarbonate (2 x 30 mL), water and saturated sodium chloride and dried over anhydrous magnesium sulfate. Evaporation
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of the solvent gave 8.01 g (100% yield) of benzyl 2-isobutyl-3-(methoxycarbonyl)-propionate (2) as a mixture of E and Z isomers.

5 A mixture of 8.01 g (0.029 mol) of (2) and 1 g of 10% palladium on carbon in 50 mL of methanol was hydrogenated at room temperature under 4 atmospheres of hydrogen gas for 8 hr. After removal of the catalyst by filtration the filtrate was evaporated to dryness under reduced pressure to give 4.7 g (86% yield) of 2-iso-
10 butyl-3-(methoxycarbonyl)-propionic acid (3) as a colorless oil.

To a mixture of 0.85 g (4.5 mmol) of (3) and 0.57 g (4.5 mmol) of oxalyl chloride in 10 mL of dry methylene chloride 0.1 mL of anhydrous dimethylformamide
15 was added. After stirring for 1 hr at room temperature the solvent was evaporated under reduced pressure and the residue was diluted to 5 mL with anhydrous dimethylformamide and 1.06 g (4.1 mmol) of the hydrochloride salt of L-tryptophan methanamide (Kortylewicz and Galardy, J Med
20 Chem (1990) 33:263-273) was added followed by addition of 1.3 mL (9.3 mmol) of triethylamine at -10°C. This was stirred for 7 hr at room temperature and evaporated to dryness at room temperature under reduced pressure. The residue was diluted to 150 mL with ethyl acetate and
25 washed with water (2 x 15 mL), 10% potassium bisulfate (5 x 20 mL), 10% sodium bicarbonate (2 x 20 mL), saturated sodium chloride and dried over anhydrous magnesium sulfate and then evaporated to give 1.6 g (83% yield) of N-[D,L-2-isobutyl-3-(methoxycarbonyl)-
30 propanoyl]-L-tryptophan methanamide 4 as a mixture of diastereomers, 4A and 4B.

Isomers 4A and 4B were separated by flash chromatography (silica gel, ethyl acetate).

Isomer 4A: mp=134-137°C. $R_f(C)=0.37$.

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Isomer 4B: mp=156-158°C. $R_f(C)=0.2$.

Alternatively, the mixture of 4A and 4B was converted directly to its hydroxamate as described below. In this case, 5A was crystallized from the mixture of 5A and 5B.

5 A warm mixture of 0.22 g (3.96 mmol) of potassium hydroxide in 1 mL of methanol was added to a warm mixture of 0.184 g (2.65 mmol) of the hydrochloride salt of hydroxylamine. After cooling in ice under an argon atmosphere the potassium chloride was filtered off and 0.5 g (1.32 mmol) of (4A) was added to the filtrate. The resulting mixture was stirred for 7 hr at room temperature and evaporated to dryness under reduced pressure. The residue was suspended in 100 mL of ethyl acetate and washed with 10 mL of 10% potassium bisulfate, saturated sodium chloride and dried over anhydrous magnesium sulfate and evaporated to dryness under reduced pressure. The residue was crystallized from ethyl acetate to give 0.28 g (56% yield) of pure 5A.

20 Isomer 4B was converted to its corresponding hydroxamic acid 5B (72% yield) as described for 4A.

Isomer 5A: mp=176-182°C. $R_f(D)=0.45$.

Isomer 5B: mp=157-162°C. $R_f(D)=0.39$.

For the case wherein the 4A/4B mixture is used, the 5A can be crystallized directly from the residue as described above.

In a similar manner to that set forth above, but substituting for 4-methyl-2-oxopentanoic acid, 2-oxopentanoic acid, 3-methyl-2-oxobutyric acid, 2-oxohexanoic acid, 5-methyl-2-oxohexanoic acid, or 2-decanoic acid, the corresponding compounds of formula 1 are prepared wherein R^1 is H and R^2 is an n-propyl, i-propyl, n-butyl, 2-methylbutyl, and n-octyl, respectively. In addition, following the procedures set forth hereinabove

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in Example 1, but omitting the step of hydrogenating the intermediate obtained by the Wittig reaction, the corresponding compounds of formula 2 wherein R^1 is H and R^2 is as set forth above are obtained.

5 To synthesize the compounds containing acylated forms of the indolyl residue, the intermediate ester of formula 3 or 4 is deesterified and acylated prior to conversion to the hydroxamate. For illustration, 4A is deesterified with sodium hydroxide in ethanol and then
10 acidified to give N-(L-2-isobutyl-3-carboxypropanoyl)-L-tryptophan methylamide, which is treated with the anhydride of an alkyl (1-4C) carboxylic acid to obtain N-(L-2-isobutyl-3-carboxypropanoyl)-L-((N-acyl)indolyl)-tryptophan methylamide. This intermediate is then
15 treated with oxalyl chloride followed by hydroxylamine at low temperature to give the corresponding hydroxamate.

Example 2

Preparation of N-[2-isobutyl-3-(N'-hydroxy-
20 carbonylamido)-propanoyl]-D-tryptophan methylamide (7B)

The mixture of the two diastereoisomers of N-[2-isobutyl-3-(methoxycarbonyl)-propanoyl]-D-tryptophan methyl amide 6A,B was prepared as described for 4A,B in Example 1. The mixture was crystallized from ethyl
25 acetate to give, after two recrystallizations, 0.26 g (49%) of the pure diastereomer 6B: mp 155-157°C, $R_f(C)=0.32$. 6B was converted into its hydroxamic acid 7B by the method described in Example 1 in 50% yield (119 mg): mp 157-159°C, $R_f(D)=0.39$.

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Example 3

Preparation of N-[2-isobutyl-3-(N'-hydroxycarbonyl-amido)-propanoyl]-N-methyl-L-tryptophan methylamide (9A)

The reaction of N-methyl-L-tryptophan-methylamide, prepared as described in Example 1 for L-tryptophan methylamide, with 3 performed as described for 4 gave crude N-[D,L-2-isobutyl-3-(methoxycarbonyl)-propanoyl]-N-methyl-L-tryptophan methylamide 8A,B which was crystallized from ethyl acetate to give 76 mg (19% yield) of 8A: mp 171-174°C, $R_f(C)=0.40$.

8A was converted into 9A by the method described in Example 1 in 45% yield (34 mg): mp 180-183°C, $R_f(D)=0.54$.

15

Example 4

Preparation of N-[2-isobutyl-3-(N-hydroxycarbonyl amido)-propanoyl]-L-3-(2-naphthyl)-alanine methylamide (11A)

N-[D,L-isobutyl-3-(methoxycarbonyl)-propanoyl]-L-3-(2-naphthyl)-alanine 10A was prepared as described in Example 1 from L-3-(2-naphthyl)-alanine methylamide and 3. The crude product was chromatographed on 60 g of silica gel in ethyl acetate:hexane 1:1 to yield 12 mg (5% yield) of 10A: mp 151-158°C, $R_f(C)=0.69$.

10A was converted into the hydroxamate 11A as in Example 1 in 30% yield (3 mg): mp 179-181°C, $R_f(D)=0.17$. MS-FAB (m/z) 400 ($M^+ + H$).

Example 5

Preparation of N-[2-isobutyl-3-(N'-hydroxycarbonyl amido)-propanoyl]-L-tryptophan 2-hydroxyethylamide (13A)

The hydrochloride salt of L-tryptophan 2-hydroxyethylamide was prepared and coupled with 3 as described for the hydrochloride salt of L-tryptophan methylamide in Example 1 except that 3 was activated with

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1,1'-carbonyldiimidazole for 20 minutes in methylene chloride at room temperature. The crude product was a mixture of 0.7 g (67% yield) of the diastereoisomers 12A,B: $R_f(C)$ 12A 0.38, $R_f(C)$ 12B 0.19.

5 12A crystallized from ethyl acetate in 35% yield (0.18 g): mp 161-163°C, $R_f(C)$ =0.38.

12A was converted into N-[2-isobutyl-3-(N'-hydroxycarbonylamido)-propanoyl]-L-tryptophan 2-hydroxy-ethylamide 13A as in Example 1 in 35% yield (62 mg):
10 $R_f(D)$ =0.17, mp 162-163°C. MS-FAB (m/z) 419 (M^+ + H).

Example 6

Preparation of N-[2-isobutyl-3-(N'-hydroxycarbonylamido)-propanoyl]-L-tryptophan amylamide (15A)

15 The hydrochloride salt of L-tryptophan amylamide was prepared as described in Example 1 for L-tryptophan methylamide and was reacted with 3 that had been activated with 1,1'-carbonyldiimidazole for 20 minutes in dichloromethane at room temperature. The
20 mixture of the two diastereomers of N-[D,L-2-isobutyl-3-(methoxycarbonyl)-propanoyl]-L-tryptophan amylamide 14A,B (90% yield) was converted to its corresponding hydroxamic acids as described for 4A. Slow evaporation of the ethylacetate solution gave 0.343 g (71%) of 15A,B: mp
25 160-163°C. MS-FAB (m/z) 445 (M^+ + H).

Example 7

Preparation of N-[2-isobutyl-3-(N'-hydroxycarbonylamido)-propanoyl]-L-tryptophan piperidinamide (17A,B)

30 L-tryptophan piperidinamide was reacted with 3 as performed in Example 1 for L-tryptophan methylamide to give 1.14 g (89% yield) of N-[D,L-2-isobutyl-3-(methoxycarbonyl)-propanoyl]-L-tryptophan piperidinamide
35 16A,B as a foam; $R_f(C)$ (16A) 0.74, (16B) 0.67.

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16A,B was converted into crude 17A,B identically to 4A in Example 1 in 88% yield (570 mg): $R_f(D)$ (17A) 0.41, (17B) 0.30. Crude 17A,B was chromatographed on 180 g of silica gel in 12% isopropanol in ethyl acetate to give 140 mg (25% yield) of 17A,B after crystallization from ethyl acetate: mp 169-170°C. MS-FAB (m/z) 443 ($M^+ + H$).

Example 8

10 Preparation of N-[2-isobutyl-3-(N'-hydroxycarbonyl
 amido)-propanoyl]-L-tryptophan dodecylamide (19A)

The reaction of L-tryptophan dodecylamide was prepared in a manner analogous to that described for L-tryptophan methylamide in Example 1. This ester was
15 reacted with 3 as described in Example 1 to give crude N-[D,L-isobutyl-3-(methoxycarbonyl)-propanol]-L-tryptophan dodecylamide 18A,B in 93% yield as a mixture of isomers 19A and 19B. This mixture was chromatographed on 150 g of silica gel in ethyl acetate:hexane, 1:2, to
20 yield 0.62 g of the mixture of the two isomers: $R_f(E)$ 19A 0.37, $R_f(E)$ 19B 0.29.

Crystallization by slow evaporation from ethyl acetate gave 0.38 g of 18A contaminated by approximately 10% of 18B by TLC and NMR analysis: mp 133-135°C. 18A
25 was converted to its corresponding hydroxamic acid as described in Example 1, except that the potassium salt of 19A crystallized from the alkaline reaction mixture in 81% yield (222 mg). The potassium salt of 19A (54 mg) was dissolved in 2 mL of boiling methanol, a few drops of
30 water were added, and the solution was acidified to pH 6 with 0.1 N hydrochloric acid and diluted with water to give 50 mg (100% yield) of 19A: mp 155-159°C, $R_f(D)=0.49$. MS-FAB (m/z) 543 ($M^+ + H$).

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Example 9Preparation of N-[2-isobutyl-3-(N'-hydroxycarbonylamido)propanoyl]-L-tryptophan (S)-methylbenzylamide (21A)

The reaction of L-tryptophan (S)-methylbenzylamide with 3 was performed as described in Example 1 to give, after crystallization from ethyl acetate, 330 mg (51% yield) of N-[2-isobutyl-3-(methoxycarbonyl)propanoyl]-L-tryptophan (S)-methylbenzylamide 20A: mp 160-162°C, $R_f(C)=0.77$.

20A was converted into hydroxamate 21A by the identical method used in Example 1 in 38% yield (76 mg): mp 165-166°C, $R_f(D)=0.73$. MS-FAB (m/z) 479 ($M^+ + H$).

Example 10

Preparation of N-[L-2-isobutyl-3-(N'-hydroxycarbonylamido)-propanoyl]-L-tryptophan (6-phenylmethoxycarbonylamino-hexyl-1)amide (27A)

To prepare 1-amino-6-phenylmethoxycarbonylamino-hexane (23), an equimolar mixture (0.01 mol) of 1,6-diaminohexane and benzaldehyde in 25 mL of methylene chloride was stirred for 5 hr in the presence of 1.5 g of anhydrous magnesium sulfate at room temperature. After removing the drying agent by filtration the filtrate was evaporated to dryness under reduced pressure to give 2 g (100% yield) of crude 1-amino-6-phenylamino-hexane 22 as a colorless oil; NMR($CDCl_3$) 1.1 - 1.9(m, 10H, hexane CH_2 -2,-3,-4,-5, NH_2); 2.6(m, 2H, CH_2 -1); 3.51(m, 2H, hexane CH_2 -6); 7.1-7.8 (m, 5H, aromatic); 8.16(s, 1H, imine CH). To a mixture of 2 g (0.01 mol) of 22 and 1.4 mL (0.01 mol) of triethylamine in 20 mL of methylene chloride. Then 1.78 g (0.01 mol) of benzylchloroformate was added dropwise at -5°C. The resulting mixture was stirred for 0.5 hr at 0°C and for 2 hr at room temperature then diluted to 50 mL with methylene chloride and

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washed with water (20 ml), 2% sodium bicarbonate (20 ml), water and saturated sodium chloride and dried over anhydrous magnesium sulfate. After evaporation of solvent under reduced pressure the residue was dissolved in 5 mL of ethanol and 10 mL of 2N hydrochloric acid was added. The resulting mixture was stirred for 6 hr at room temperature then evaporated to dryness under reduced pressure. The residue was diluted to 50 mL with water and washed with ethyl ether (2 x 15 ml). The water phase was evaporated under reduced pressure and the product 23 was purified by crystallization from a small portion of water with a yield of 42%; mp 175-178°C.

To prepare the dipeptide analog (N-(L-2-isobutyl-3-methoxycarbonyl)-propanoyl-L-tryptophan (25A)), for derivatization to 23: To a mixture of 1.754 g (9.32 mmol) of 2-isobutyl-3-methoxycarbonylpropionic acid 3 in 4 mL of 50% anhydrous DMF in methylene chloride 1.66 g (10.2 mmol) of N,N'-carbonyldiimidazole (CDI) was added at room temperature. After 15 minutes of stirring at room temperature, 3.08 g (9.31 mmol) of the hydrochloride salt of L-tryptophan benzyl ester was added. The resulting mixture was stirred overnight at room temperature, then diluted to 60 mL with ethyl acetate and washed with 5% sodium bicarbonate (2 x 15 ml), water (2 x 15 ml), saturated sodium chloride solution and dried over magnesium sulfate. Evaporation of the solvent under reduced pressure gave 4.32 g (100% yield) of 24, the benzyl ester of 25 as a colorless foam, which was used in the next step without further purification.

Hydrogen gas was bubbled through a mixture of 4.32 g (9.31 mmol) of 24 and 0.5 g of 10% palladium on carbon in 15 mL of methanol for 2 hr while methanol was added to keep the volume of the reaction mixture constant. The catalyst was filtered off and washed with

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a fresh portion of methanol (15 ml) and the filtrate was evaporated to dryness under reduced pressure. Evaporation of the solvent under reduced pressure and drying of the residue in vacuo gave 3.08 g (88% yield) of acid
5 25A,B as a mixture of two diastereoisomers, in the form of a colorless glassy solid. This was separated to give isomers 25A and 25B by flash chromatography (silica gel; ethyl acetate; $R_f(\text{25A})=0.24$, $R_f(\text{25B})=0.1$).

The compound 25A was converted to N-[L-2-
10 isobutyl-3-methoxycarbonylpropanoyl]-L-tryptophan (6-phenylmethoxycarbonylamino-hexyl-1)amide (26A) as follows. A mixture of 0.55 g (1.47 mmol) of 25A and 0.24 g (1.48 mmol) of CDI in 1 mL of 2% dimethylformamide in methylene chloride was stirred for 0.5 hr at room
15 temperature and 0.42 g (1.47 mmol) of 23 was added. After stirring overnight at room temperature, the mixture was diluted to 50 mL with chloroform and washed with 2% potassium bisulfate (2 x 10 mL), water (10 mL), 5% sodium bicarbonate (2 x 10 mL), water (2 x 10 mL) and saturated
20 sodium chloride and dried over anhydrous magnesium sulfate. Evaporation of the solvent under reduced pressure gave 0.8 g of the crude 26A which was purified by flash chromatography (silica gel; ethyl acetate/hexane 25:5): Yield 56%; $R_f(E)=0.57$.

25 When the product 26A is substituted for 4A in Example 1, the identical process afforded the title compound 27A, melting at 102-108°C, in 46% yield; $R_f(D)=0.63$.

30

Example 11

Preparation of N-[L-2-isobutyl-3-(N'-hydroxycarbonylamido)-propanoyl]-L-tryptophan cyclohexylamide (28A)

When cyclohexylamine is substituted for 23 in
35 Example 10, the identical process afforded the title

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compound 28A melting at 199-203°C, in 49% yield;
 $R_f(D)=0.51$.

Example 12

5 Preparation of N-[cis-2-(N'-hydroxycarbonyl-
amido)-cyclohexylcarbonyl]-L-tryptophan methylamide (29A,B)

A mixture of 2 g (0.013 mol) of cis-1,2-cyclohexane-dicarboxylic anhydride in 15 mL of methanol was refluxed for 5 hr, then evaporated to dryness under
10 reduced pressure to give 2.41 g (100% yield) of cis-2-methoxycarbonyl-cyclohexanecarboxylic acid. When this was substituted for 3 in Example 1, the identical process afforded the title compound, melting at 140-144°C, in 36% yield; $R_f(D)=0.53, 0.47$.

15

Example 13

Preparation of N-[trans-2-(N'-hydroxycarbonyl-
amido)-cyclohexylcarbonyl]-L-tryptophan methylamide (30A,B)

When (\pm)trans-1,2-cyclohexanedicarboxylic
20 anhydride was substituted for cis-1,2-cyclohexane-dicarboxylic anhydride in Example 12, the identical process afforded the title compound 30A,B, melting at 167-174°C, in 37% yield; $R_f(D)=0.57$.

25

Example 14

Preparation of N-[2-isobutyl-3-(N'-hydroxycarbonyl-
amido)-propanoyl]-L-tryptophan (31A)

31A was prepared from 25A in Example 10 in a similar manner to the preparation of 5A in Example 1 in
30 75% yield (128 mg) and isolated as a foam from ethyl acetate: $R_f(F)=0.55$, MS-FAB (m/z) ($M^+ + H$). A small sample of 31A recrystallized from ethyl acetate had a melting point of 116-120°C.

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Example 15Preparation of N-(D,L-2-isobutyl-3-carboxypropanoyl)-L-tryptophan (6-aminohexyl-1)amide (32A)

A mixture of 0.5 g (8.24 mmol) of 26A in 0.4 mL of 2N potassium hydroxide in methanol was stirred overnight at room temperature, then evaporated to dryness under reduced pressure. The residue was diluted to 15 mL with water and acidified to pH = 2 with 1N hydrochloric acid. The crude free acid of 26A was taken up with ethyl acetate (3 x 15 ml) and the organic phase was dried over anhydrous magnesium sulfate and evaporated to dryness to give 0.45 g (92% yield) of 26A as a colorless foam.

Hydrogen gas was bubbled through a mixture of 0.395 g (6.6 mmol) of the free acid of 26A in 15 mL of methanol for 2 hr, in the presence of 0.12 g of 10% palladium on carbon at room temperature. The catalyst was filtered off, washed with ethanol (2 x 20 ml) and the filtrate was evaporated to dryness under reduced pressure to give 0.3 g (92% yield) of the title compound 32A as a colorless foam; $R_f(G) = 0.08$.

Example 16Preparation of N-[N-(2-isobutyl-3-carboxypropanoyl)-L-tryptophanyl] glycine 34A,B

The reaction of L-tryptophanyl-glycine methyl ester with acid 3, performed as described for 25A gave crude N-[N-(D,L-2-isobutyl-3-methoxycarbonylpropanoyl)-L-tryptophanyl]-glycine methyl ester 33 in 87% yield as a mixture of diastereomers 33A and 33B. Isomers 33A and 33B were separated by flash chromatography (silica gel; ethyl acetate). Isomer 33A mp = 154-155°C; $R_f(C) = 0.46$.

Esters 33A,B were transformed to free acids 34A,B by saponification with two equivalent of methanolic

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potassium hydroxide, as described for 25A. Isomer 34A yield 92%; mp = 96-102°C; $R_f(G)$ = 0.31.

Isomer 34B yield 93%; mp = 99-105°C;
 $R_f(G)$ = 0.25.

5

Example 17

Preparation of N-(cis-2-carboxy-cyclohexylcarbonyl)- L-tryptophan methylamide 35

To a mixture of 0.281 g (1.82 mmol) of cis-
10 1,2-cyclohexanedicarboxylic anhydride and 0.47 g of the
hydrochloride salt of L-Trp-NHMe in 0.5 mL of dimethyl-
formamide 0.51 mL of triethylamine was added at room
temperature. After 2 hr of stirring the resulting
mixture was diluted to 10 mL with water and 25 mL of
15 ethyl acetate was added. The resulting mixture was
acidified to pH = 2 with 10% potassium bisulfate and the
organic phase was washed with water (2 x 15 mL),
saturated sodium chloride and dried over anhydrous
magnesium sulfate and evaporated to dryness. The title
20 compound 35 was purified by crystallization from an ethyl
acetate-hexane mixture. Yield 48%; mp = 105-112°C;
 $R_f(G)$ = 0.65, 0.61.

Example 18

Preparation of N-(trans-2-carboxy-cyclohexylcarbonyl)- L-tryptophan methylamide 36

When (+) trans-1,2-cyclohexanedicarboxylic
anhydride is substituted for cis-1,2-cyclohexane-
dicarboxylic anhydride in Example 17, the identical
30 process afforded the title compound 36 in 56% yield:
mp = 167-174°C; $R_f(G)$ = 0.67, 0.61.

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Example 19Preparation of N-[2-isobutyl-3-(N'-acetoxycarbonylamido)-propanoyl]-L-tryptophan methylamide (37A)

To 97.5 mg (0.25 mmol) of 5A (Example 1) in 0.5 ml of dimethylformamide was added 25.5 mg (0.25 mmol) of acetic anhydride and 37 mg (0.25 mmol) of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) at room temperature. After standing overnight, the DMF was evaporated under high vacuum and the residue taken up in a mixture of equal volumes of ethyl acetate and 2% potassium bisulfate. The ethyl acetate layer was washed with 2% potassium bisulfate, water, and brine, dried over magnesium sulfate, and evaporated to give a solid. The solid was dissolved in a 1:1 mixture of hot ethyl acetate:hexane, which upon standing at room temperature gave 71 mg (66% yield) of solid product 37A: mp=184-186°C; Rf(G)=0.68.

Example 20

20 Preparation of N-[isobutyl-3-(N'-benzoxycarbonylamido)-propanoyl]-L-tryptophan methylamide (38A)

To 30.5 mg (0.25 mmol) of benzoic acid in 1 ml of tetrahydrofuran was added 40.5 mg (0.25 mmol) of carbonyldiimidazole. After 10 minutes, 97 mg (0.25 mmol) of compound 5A from Example 1 was added in 1 ml of dimethylformamide. After 10 minutes, the reaction mixture was evaporated to dryness under high vacuum, and dissolved in a mixture of equal volumes of ethyl acetate and water. The ethyl acetate layer was washed with 5% sodium bicarbonate, water, 2% sodium bisulfate, water, and brine, and dried over magnesium sulfate. Evaporation of the ethyl acetate layer to a small volume gave 50 mg (41%) of the title compound, 38A: mp=187-187.5°C; Fr(G)=0.54.

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Example 21

Applying the methods set forth above, the following invention compounds are synthesized.

- 5 EtONHCONMe-CH₂CH(iBu)-CO-L-Trp-NHEt
 EtCONOH-CH₂CH(iBu)-CO-L-Trp-NHEt
 n-PrCONOEt-CH₂CH(iBu)-CO-L-Trp-NHEt
 EtNHCONOMe-CH₂CH(iBu)-CO-L-Trp-NHEt
 MeNHCONOH-CH₂CH(iBu)-CO-L-Trp-NHEt
 10 EtONHCONMe-CH₂CH(iBu)-CO-L-Ala(2-naphthyl)-NHEt
 EtCONOH-CH₂CH(iBu)-CO-L-Ala(2-naphthyl)-NHEt
 n-PrCONOEt-CH₂CH(iBu)-CO-L-Ala(2-naphthyl)-NHEt
 EtNHCONOMe-CH₂CH(iBu)-CO-L-Ala(2-naphthyl)-NHEt
 MeNHCONOH-CH₂CH(iBu)-CO-L-Ala(2-naphthyl)-NHEt
 15 HONHCONHCH₂CH(iBu)-CO-L-TrpNHMe
 HONHCONHCH₂CH₂CH(iBu)-CO-L-TrpNHMe
 HONHCONHCH(iBu)CO-L-TrpNHMe
 H₂NCON(OH)CH(iBu)CO-L-TrpNHMe
 H₂NCON(OH)CH₂CH(iBu)CO-L-TrpNHMe
 20 H₂NCON(OH)CH₂CH₂CH(iBu)CO-L-TrpNHMe
 CH₃CON(OH)CH(iBu)CO-L-TrpNHMe
 CH₃CON(OH)CH₂CH(iBu)CO-L-TrpNHMe
 CH₃CON(OH)CH₂CH₂CH(iBu)CO-L-TrpNHMe

25

Example 22Assay of Inhibition Activity

- Inhibitors were assayed against crude or purified human skin fibroblast collagenase using the synthetic thiol ester substrate at pH 6.5 exactly as
 30 described by Kortylewicz & Galardy, J Med Chem (1990) 33:263-273. The collagenase concentration was 1-2 nM. The compounds of Examples 1-18 are tested for their ability to inhibit crude collagenase and gelatinase from human skin fibroblasts, crude collagenase and gelatinase
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-40-

from purulent human sputum in this assay. The results with respect to crude enzyme preparation are shown in Table 1. The K_i of 5A for purified human skin collagenase is 0.4 nM. Assays for inhibition of human stromelysin are conducted as described by Teahan, J., et al., Biochemistry (1989) 20:8497-8501.

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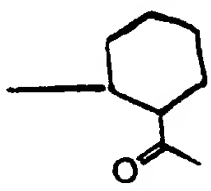
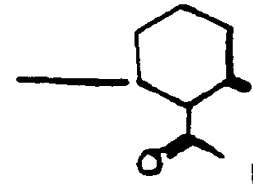
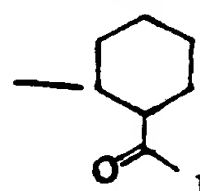
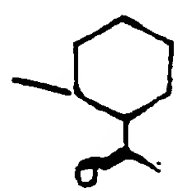
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Table 1

No.	Compound	K _i (nM)		
1	5A	NHOHCOCH ₂ CH(i-Bu)CO-L-Trp-NHMe	10	
1	5B	NHOHCOCH ₂ CH(i-Bu)CO-L-Trp-NHMe	150	
5	2	7A	NHOHCOCH ₂ CH(i-Bu)CO-D-Trp-NHMe	70,000
3	9A	NHOHCOCH ₂ CH(i-Bu)CO-L-N-MeTrp-NHMe	500	
4	11A	NHOHCOCH ₂ CH(i-Bu)CO-L-Ala(2-naphthyl)NHMe	15	
5	13A	NHOHCOCH ₂ CH(i-Bu)CO-L-Trp-NH(CH ₂) ₂ OH	20	
6	15A	NHOHCOCH ₂ CH(i-Bu)CO-L-Trp-NH(CH ₂) ₄ CH ₃	30	
10	7	17A, B	NHOHCOCH ₂ CH(i-Bu)CO-L-Trp-piperidine	200
8	19A	NHOHCOCH ₂ CH(i-Bu)CO-L-Trp-NH(CH ₂) ₁₁ CH ₃	300	
9	21A	NHOHCOCH ₂ CH(i-Bu)CO-L-Trp-NH(S)CHMePh	3	
10	27A	NHOHCOCH ₂ CH(i-Bu)CO-L-Trp-NH(CH ₂) ₆ NH-CBZ	13	
11	28A	NHOHCOCH ₂ CH(i-Bu)CO-L-Trp-NHcyclohexyl	50	
15	12	29A, B	cis-NHOHCO  L-Trp-NHMe	>10,000
20	13	30A, B	trans-NHOHCO  L-Trp-NHMe	>10,000
14	31A	NHOHCO-CH ₂ CH(i-Bu)-L-Trp-OH	200	
15	32A	HOOC-CH ₂ CH(i-Bu)CO-L-Trp-NH(CH ₂)NH ₂	>10,000	
25	16	34A	HOCO-CH ₂ CH(i-Bu)CO-L-Trp-Gly-OH	>10,000
		34B	HOCO-CH ₂ CH(i-Bu)CO-L-Trp-Gly-OH	>10,000
17	35	cis-HOCO  L-Trp-NHMe	>10,000	
30				
18	36	trans-HOCO  L-Trp-NHMe	>10,000	
35				

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Example 21
Prevention of Corneal Ulceration in the
Alkali Burned Rabbit Cornea

5 The ability of the invention compounds to prevent ulceration has been confirmed by a corneal assay conducted by Gregory Schultz, University of Florida, Gainesville, FL.

10 Twenty rabbit eyes were burned for 60 seconds with 1 N NaOH to a diameter of 10 mm. The ten control eyes were treated with two drops of hypotonic buffer every two hours from 8 AM to 6 PM, and then with a subconjunctival injection of 0.5 mL of buffer in the evening. The experimental eyes were treated identically but with 400 µg per mL inhibitor in buffer. Eyes were scored
15 clinically with 0 being no ulceration and 5 equalling perforation of the cornea. Figure 1 shows average clinical score over 26 days. The compound 5A shows marked protection of the cornea from perforation. Figure 2 shows percent of corneas not perforated over 26 days;
20 compound 5A shows 100% protection.

A histological examination of the treated and untreated corneas showed the following:

25 Perpendicular sections through the corneas of rabbits were examined 28 days following severe alkali injuries which were made by exposing the corneas of anesthetized rabbits to 2 N sodium hydroxide for 60 seconds in a 12.5 mm diameter well. Following injury, rabbits were treated topically with 2 drops every other hour between 8 AM and 6 PM followed by a subconjunctival injection of 0.5 mL of
30 collagenase inhibitor of formula 5A or vehicle (50 mM Hepes, Antibiotics).

The cornea of a rabbit treated with collagenase inhibitor shows lamellae that have begun to repopulate with keratocytes which have most likely migrated from the
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uninjured peripheral cornea and sclera. The stroma also contains some inflammatory cells presumably macrophages which have migrated into the injured cornea. There is little evidence for disruption of the extracellular matrix of the stromal lamellae and there is no evidence of significant extracellular matrix destruction. The epithelium in this section has resurfaced the cornea, although the epithelium is tenuously attached as evidenced by the separation of the sections of the epithelium from the underlying stroma. There is no evidence of neovascularization of this part of the cornea. The endothelial surface has not regenerated and Descemet's membrane has separated from the stroma in a cloudy cornea which lacks persistent and complete epithelial regeneration and also lacks significant stromal ulceration.

Cornea treated only with vehicle shows a dominant appearance of extensive degradation of the stromal matrix and the presence of massive inflammatory cell infiltrates. These inflammatory cells most probably are macrophages and neutrophils. The stromal lamellae have digested in this section approximately two-thirds of the complete depth of the stroma, and in adjacent sections, erosion has occurred to Descemet's membrane. The stroma appears to have a frayed appearance at the edge where the inflammatory cell infiltration is most extensive. Fracture lines running through the stroma suggest a general weakening of the extracellular matrix. In this section of the cornea there is no evidence of neovascularization, epithelial cells, or endothelial cells. Fragments of endothelial cells are present on Descemet's membrane together with inflammatory cells in the anterior chamber fluid. Few if any keratocytes can be identified in the stroma. This microscopic section is generally

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consistent with the results of slit lamp microscopy which indicated that the cornea had extensive ulceration and peripheral neovascularization.

Overall, the histopathology of these two sections
5 suggest that a major effect of the collagenase inhibitor
in preventing ulceration is due to a reduction in the
inflammatory cell filtration into the alkali injured
cornea. Furthermore, this section suggests that repopu-
lation of the stroma in the collagenase inhibitor treated
10 corneas has begun and incomplete epithelial regeneration
of a transient nature has begun on the epithelial surface
with no regeneration of the endothelium.

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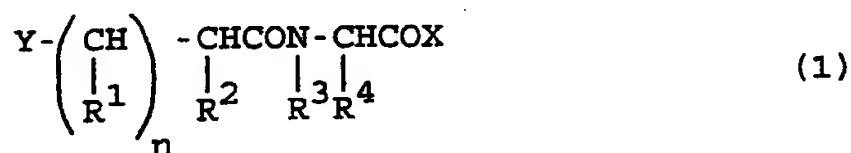
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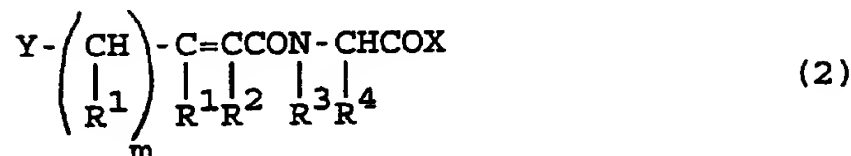
Claims

1. A compound which inhibits at least one mammalian matrix metalloprotease, said compound having the formula:



10

or



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wherein each R^1 is independently H or alkyl (1-8C) and R^2 is alkyl (1-8C) or wherein the proximal R^1 and R^2 taken together are $-(\text{CH}_2)_p-$ wherein $p = 3-5$;

R^3 is H or alkyl (1-4C);

20

R^4 is fused or conjugated unsubstituted or substituted bicycloaryl methylene;

n is 0, 1 or 2;

m is 0 or 1; and

25

X is OR^5 or NHR^5 , wherein R^5 is H or substituted or unsubstituted alkyl (1-12C), aryl (6-12C), aryl alkyl (6-16C); or

X is an amino acid residue or amide thereof; or

X is the residue of a cyclic amine or heterocyclic amine;

30

Y is selected from the group consisting of $\text{R}^7\text{ONR}^6\text{CONR}^6-$, $\text{R}^6_2\text{NCONOR}^7-$, and $\text{R}^6\text{CONOR}^7-$, wherein each R^6 is independently H or lower alkyl (1-4C); R^7 is lower alkyl (1-4C) or an acyl group; and

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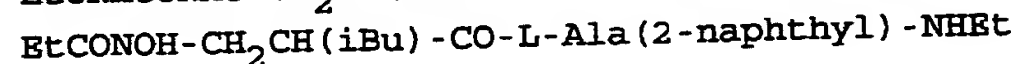
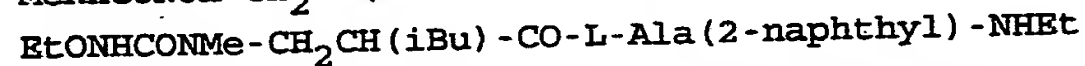
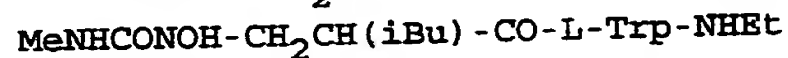
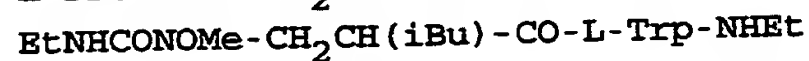
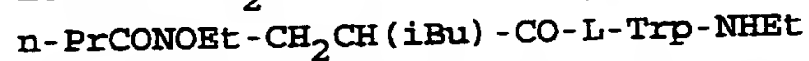
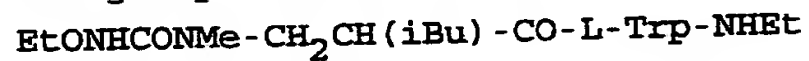
-46-

wherein $-\text{CONR}^3-$ is optionally in modified isosteric form.

2. The compound of claim 1 wherein R^3 is H, and/or all R^6 are H and R^7 is H or acyl, and/or R^1 is H and/or R^2 is alkyl (3-8C) and/or X is NHR^5 or the residue of a cyclic amine or heterocyclic amine, and/or R^4 is selected from the group consisting of 1-(2-methylnaphthyl)methylene; 1-quinolyl methylene; 1-naphthyl methylene; 2-naphthyl methylene; 1-isoquinolyl methylene; 3-isoquinolyl methylene; 3-thionaphthenyl methylene; 3-cumaronyl methylene; 3-(5-methylindolyl)methylene; 3-(5-hydroxyindolyl)methylene; 3-(2-hydroxyindolyl)methylene; biphenyl methylene; (3-indolyl)methylene; and 4-phenylpyrimidyl methylene; and the substituted forms thereof, and/or $n=1$.

3. The compound of claim 2 wherein R^2 is isobutyl, 2-methylbutyl, or isopropyl, and/or X is NHR^5 and wherein R^5 is alkyl (1-4C); a substituted alkyl (1-4C substituted with one hydroxyl group); aryl alkyl; or a substituted alkyl (2-7C substituted with phenylmethoxycarbonylamido), and/or R^4 is (3-indolyl)methylene or its N-acylated (1-5C) substituted form.

4. The compound of claim 1 which is selected from the group consisting of:



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n-PrCONOEt-CH₂CH(iBu)-CO-L-Ala(2-naphthyl)-NH₂
 EtNHCONOMe-CH₂CH(iBu)-CO-L-Ala(2-naphthyl)-NH₂
 MeNHCONOH-CH₂CH(iBu)-CO-L-Ala(2-naphthyl)-NH₂
 HONHCONHCH₂CH(iBu)-CO-L-TrpNHMe;
 5 HONHCONHCH₂CH₂CH(iBu)-CO-L-TrpNHMe;
 HONHCONHCH(iBu)CO-L-TrpNHMe;
 H₂NCON(OH)CH(iBu)CO-L-TrpNHMe;
 N(OH)CH₂CH(iBu)CO-L-TrpNHMe;
 H₂NCON(OH)CH₂CH₂CH(iBu)CO-L-TrpNHMe;
 10 CH₃CON(OH)CH(iBu)CO-L-TrpNHMe;
 CH₃CON(OH)CH₂CH(iBu)CO-L-TrpNHMe; and
 CH₃CON(OH)CH₂CH₂CH(iBu)CO-L-TrpNHMe.

5. The compound of claim 1-4 conjugated to a
 15 moiety which is a radioactive label, an antigenically
 neutral carrier, a targeting ligand, or a solid support.

6. A pharmaceutical composition effective in
 treating conditions characterized by unwanted matrix
 20 metalloprotease activity, which composition comprises an
 amount of the compound of claim 1-4 effective to inhibit
 said matrix metalloprotease activity in admixture with a
 pharmaceutically acceptable excipient.

25 7. The use of the compound of claim 1-4 or a
 pharmaceutical composition thereof to treat conditions
 characterized by unwanted matrix metalloprotease
 activity, wherein said condition is selected from the
 group consisting of stomach ulcers, superficial wounds,
 30 epidermolysis bullosa, skin cancer, pemphigus, septic
 shock and ARDS.

8. A method to inhibit matrix metalloprotease
 activity in vitro or in cell culture, which method
 35

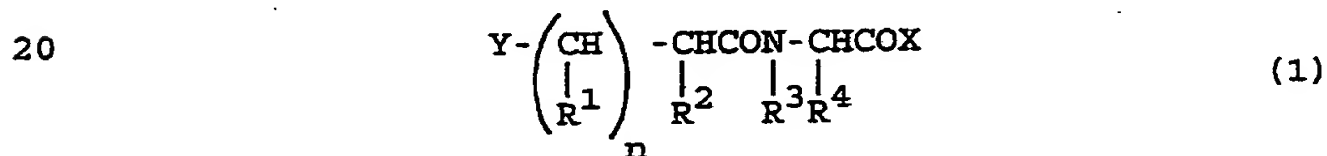
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comprises contacting the matrix metalloprotease to be inhibited with an effective amount of the compound of claim 1-4.

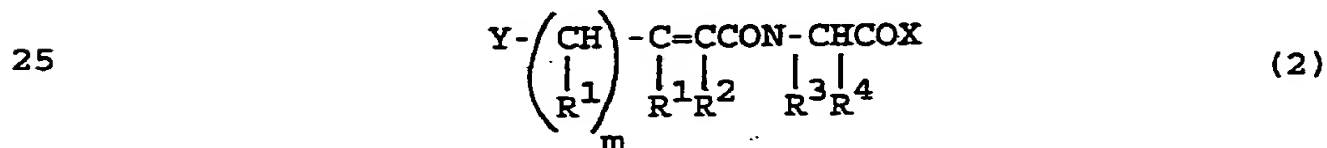
5 9. An antibody preparation specifically immunoreactive with the compound of claim 1-4.

10 10. A method to assess the level of the compound of claim 1-4 in a biological sample, which method comprises contacting the sample with an antibody preparation specifically immunoreactive with the compound of claim 1-4 under conditions wherein said antibodies complex with said compound if present, and
15 detecting the presence or absence of the complex.

11. A method to prepare a compound of the formula:



or



30 wherein each R^1 is independently H or alkyl (1-8C) and R^2 is alkyl (1-8C) or wherein the proximal R^1 and R^2 taken together are $-(\text{CH}_2)_p-$ wherein $p = 3-5$;

R^3 is H or alkyl (1-4C);

R^4 is fused or conjugated unsubstituted or substituted bicycloaryl methylene;

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n is 0, 1 or 2;

m is 0 or 1; and

X is OR⁵ or NHR⁵, wherein R⁵ is H or substituted or unsubstituted alkyl (1-12C), aryl (6-12C), aryl alkyl (6-16C); R⁷ is lower alkyl (1-4C) or an acyl group, or

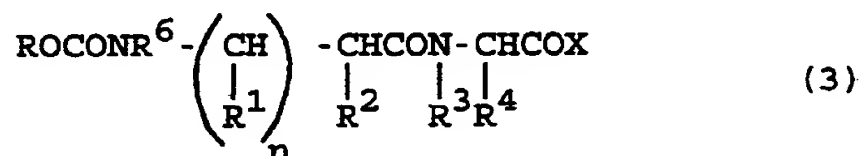
X is an amino acid residue or amide thereof; or

X is the residue of a cyclic amine or heterocyclic amine;

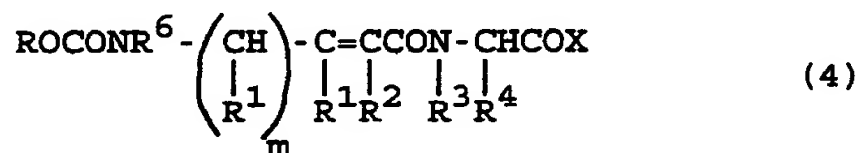
Y is R⁷ONR⁶CONR⁶-, wherein each R⁶ is independently H or lower alkyl (1-4C); R⁷ is lower alkyl (1-4C) or an acyl group; and

wherein -CONR³- is optionally in modified isosteric form,

which method comprises reacting a compound of the formula:



or



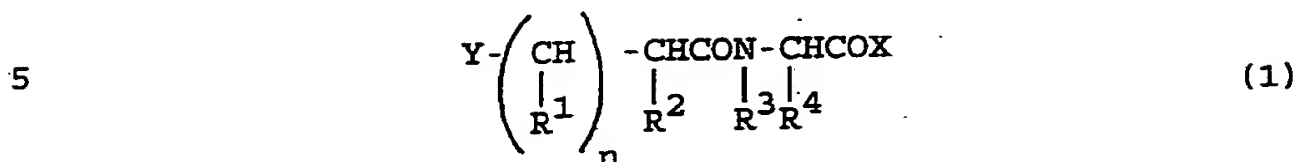
wherein R is H or alkyl and the remaining substituents are as above defined, with a compound of the formula



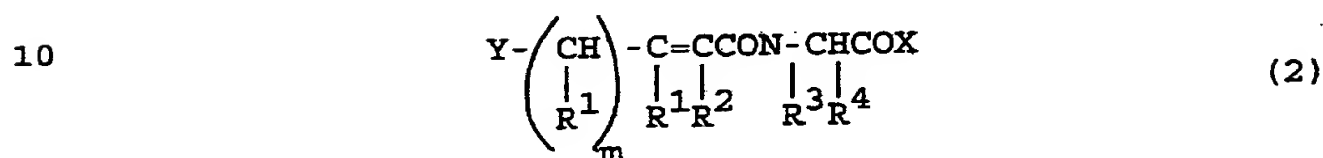
wherein R⁷ and R⁶ are as above defined under conditions which permit the formation of the compound of formula 1 from the compound of formula 3 and of formula 2 from the compound of formula 4.

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12. A method to prepare a compound of the formula:



or



15 wherein each R^1 is independently H or alkyl (1-8C) and R^2 is alkyl (1-8C) or wherein the proximal R^1 and R^2 taken together are $-(\text{CH}_2)_p-$ wherein $p = 3-5$;

R^3 is H or alkyl (1-4C);

R^4 is fused or conjugated unsubstituted or substituted bicycloaryl methylene;

20 n is 0, 1 or 2;

m is 0 or 1; and

X is OR^5 or NHR^5 , wherein R^5 is H or substituted or unsubstituted alkyl (1-12C), aryl (6-12C), aryl alkyl (6-16C); or

25 X is an amino acid residue or amide thereof; or

X is the residue of a cyclic amine or heterocyclic amine;

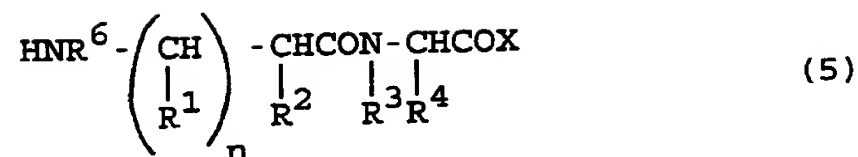
Y is $\text{R}^7\text{ONR}^6\text{CONR}^6-$, wherein each R^6 is independently H or lower alkyl (1-4C); R^7 is lower alkyl (1-4C) or an acyl group; and

30 wherein $-\text{CONR}^3-$ is optionally in modified isosteric form,

which method comprises reacting a compound of the formula:

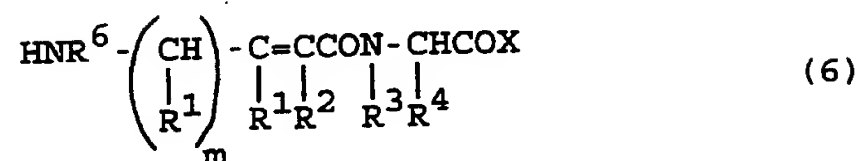
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- 51 -



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or



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wherein the indicated substituents are as above defined,

with a carbonylating agent followed by reaction with a compound of the formula

15



wherein in R^7 and R^6 are as above defined,

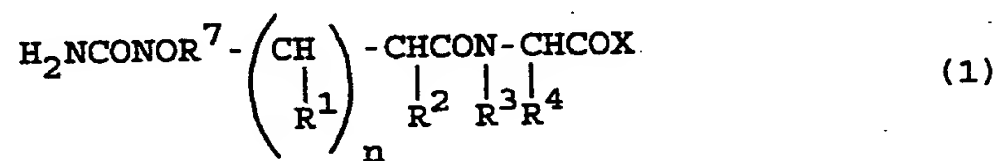
under conditions which permit the formation of the compound of formula 1 from the compound of formula 5 and the compound of formula 2 from the compound of

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formula 6.

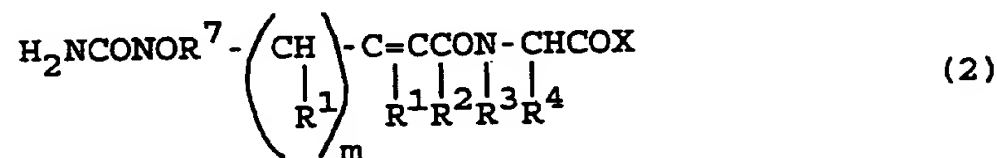
13. A method to prepare a compound of the formula:

25



or

30



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wherein each R^1 is independently H or alkyl (1-8C) and R^2 is alkyl (1-8C) or wherein the proximal R^1 and R^2 taken together are $-(CH_2)_p-$ wherein $p = 3-5$;

R^3 is H or alkyl (1-4C);

5 R^4 is fused or conjugated unsubstituted or substituted bicycloaryl methylene;

n is 0, 1 or 2;

m is 0 or 1; and

10 X is OR^5 or NHR^5 , wherein R^5 is H or substituted or unsubstituted alkyl (1-12C), aryl (6-12C), aryl alkyl (6-16C); or

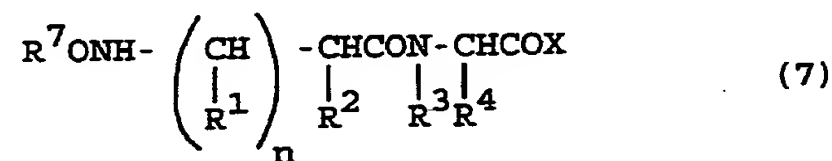
X is an amino acid residue or amide thereof; or

X is the residue of a cyclic amine or heterocyclic amine; and

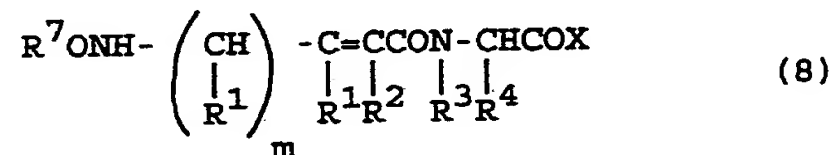
15 each R^6 is independently H or lower alkyl (1-4C); R^7 is lower alkyl (1-4C) or an acyl group; and

wherein $-CONR^3-$ is optionally in modified isosteric form,

20 which method comprises reacting a compound of the formula:



25 or



30 wherein the indicated substituents are as above defined,

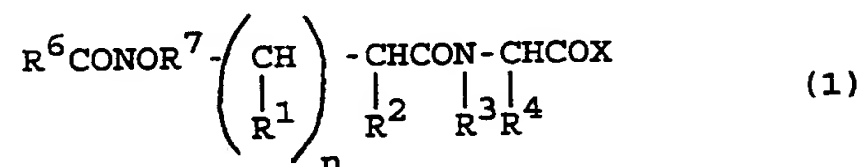
with silicon tetraisocyanate under conditions wherein the compound of formula 1 is obtained from the

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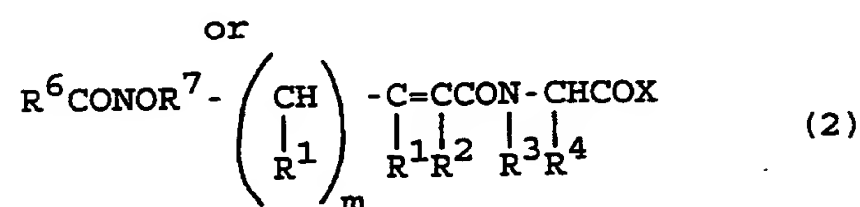
- 53 -

compound of formula 7 or the compound of formula 2 is obtained from the compound of formula 8.

14. A method to prepare a compound of the
5 formula:



10



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wherein each R^1 is independently H or alkyl (1-8C) and R^2 is alkyl (1-8C) or wherein the proximal R^1 and R^2 taken together are $-(CH_2)_p-$ wherein $p = 3-5$;

R^3 is H or alkyl (1-4C);

20

R^4 is fused or conjugated unsubstituted or substituted bicycloaryl methylene;

n is 0, 1 or 2;

m is 0 or 1; and

25

X is OR^5 or NHR^5 , wherein R^5 is H or substituted or unsubstituted alkyl (1-12C), aryl (6-12C), aryl alkyl (6-16C); or

X is an amino acid residue or amide thereof; or

X is the residue of a cyclic amine or heterocyclic amine;

30

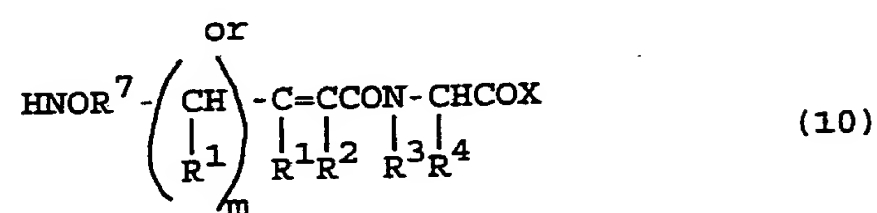
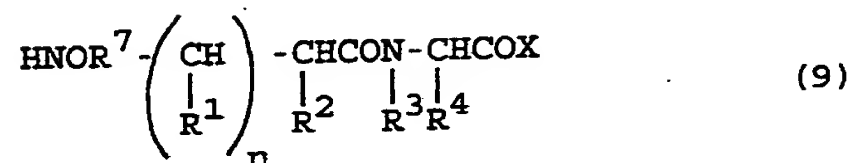
each R^6 is independently H or lower alkyl (1-4C); R^7 is lower alkyl (1-4C) or an acyl group; and

wherein $-CONR^3-$ is optionally in modified isosteric form,

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-54-

which method comprises reacting a compound of the formula:

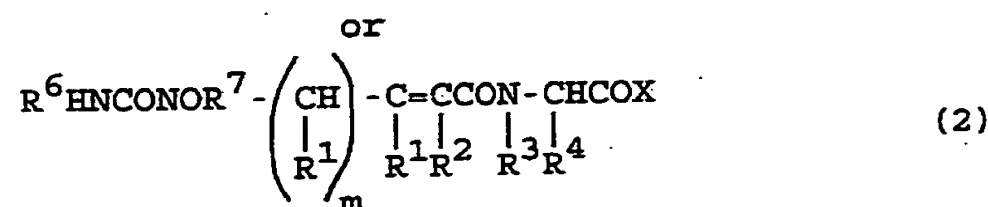
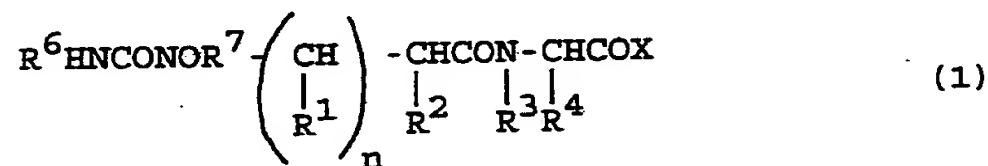


wherein the indicated substituents are as above defined,

with the compound of the formula,
 $\text{R}^6\text{CO-L}$,

wherein L is a leaving group and R^6 is as above defined under conditions wherein the compound of formula 1 is obtained from the compound of formula 9 and the compound of formula 2 is obtained from the compound of formula 10.

15. A method to prepare a compound of the formula:



-55-

wherein each R^1 is independently H or alkyl (1-8C) and R^2 is alkyl (1-8C) or wherein the proximal R^1 and R^2 taken together are $-(CH_2)_p-$ wherein $p = 3-5$;

R^3 is H or alkyl (1-4C);

5 R^4 is fused or conjugated unsubstituted or substituted bicycloaryl methylene;

n is 0, 1 or 2;

m is 0 or 1; and

10 X is OR^5 or NHR^5 , wherein R^5 is H or substituted or unsubstituted alkyl (1-12C), aryl (6-12C), aryl alkyl (6-16C); or

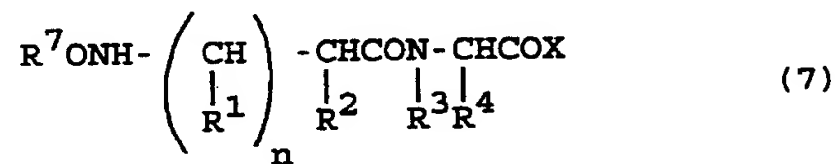
X is an amino acid residue or amide thereof; or

X is the residue of a cyclic amine or heterocyclic amine; and

15 R^6 is lower alkyl (1-4C); R^7 is lower alkyl (1-4C) or an acyl group; and

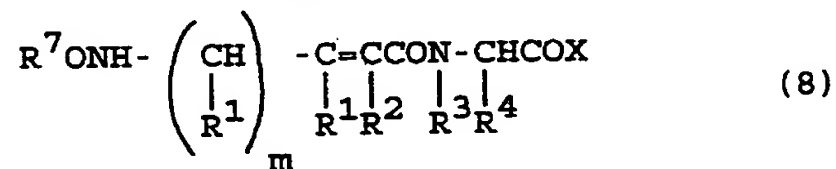
wherein $-CONR^3-$ is optionally in modified isosteric form,

20 which method comprises reacting a compound of the formula:



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or



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wherein the indicated substituents are as above defined,

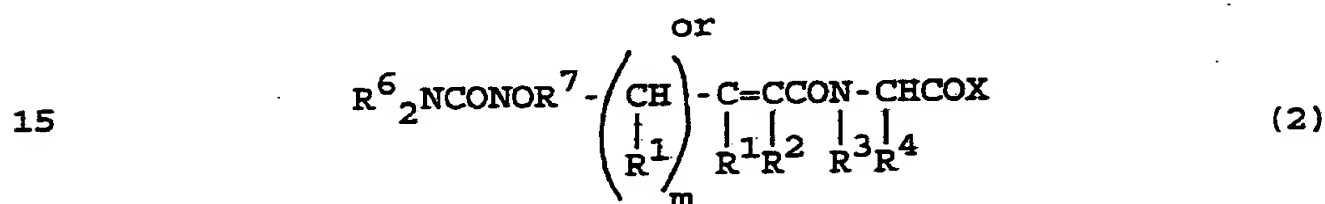
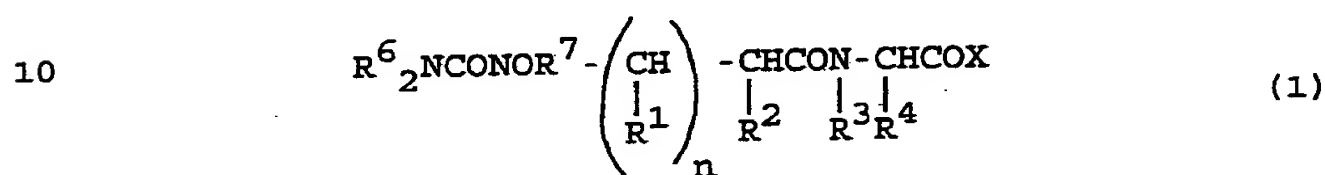
with a compound of the formula,
 R^6NCO ,

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-56-

wherein R^6 is alkyl(1-4C),
 under conditions wherein the compound of
 formula 1 is obtained from the compound of formula 7 or
 the compound of formula 2 is obtained from the compound
 5 of formula 8.

16. A method to prepare a compound of the
 formula:



wherein each R^1 is independently H or alkyl (1-
 8C) and R^2 is alkyl (1-8C) or wherein the proximal R^1 and
 20 R^2 taken together are $-(CH_2)_p-$ wherein $p = 3-5$;

R^3 is H or alkyl (1-4C);

R^4 is fused or conjugated unsubstituted or
 substituted bicycloaryl methylene;

25 n is 0, 1 or 2;

m is 0 or 1; and

X is OR^5 or NHR^5 , wherein R^5 is H or substi-
 tuted or unsubstituted alkyl (1-12C), aryl (6-12C), aryl
 alkyl (6-16C); or

30 X is an amino acid residue or amide thereof; or

X is the residue of a cyclic amine or hetero-
 cyclic amine; and

each R^6 is independently lower alkyl (1-4C); R^7
 is lower alkyl(1-4C) or an acyl group; and

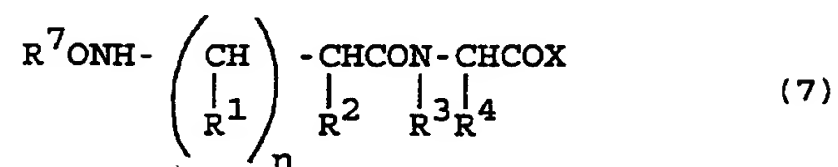
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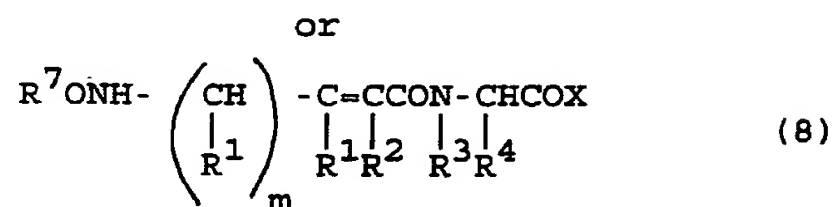
wherein $-\text{CONR}^3-$ is optionally in modified isosteric form,

which method comprises reacting a compound of the formula:

5



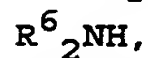
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wherein the indicated substituents are as above defined,

with an activated form of carbonic acid and then with a compound of the formula,



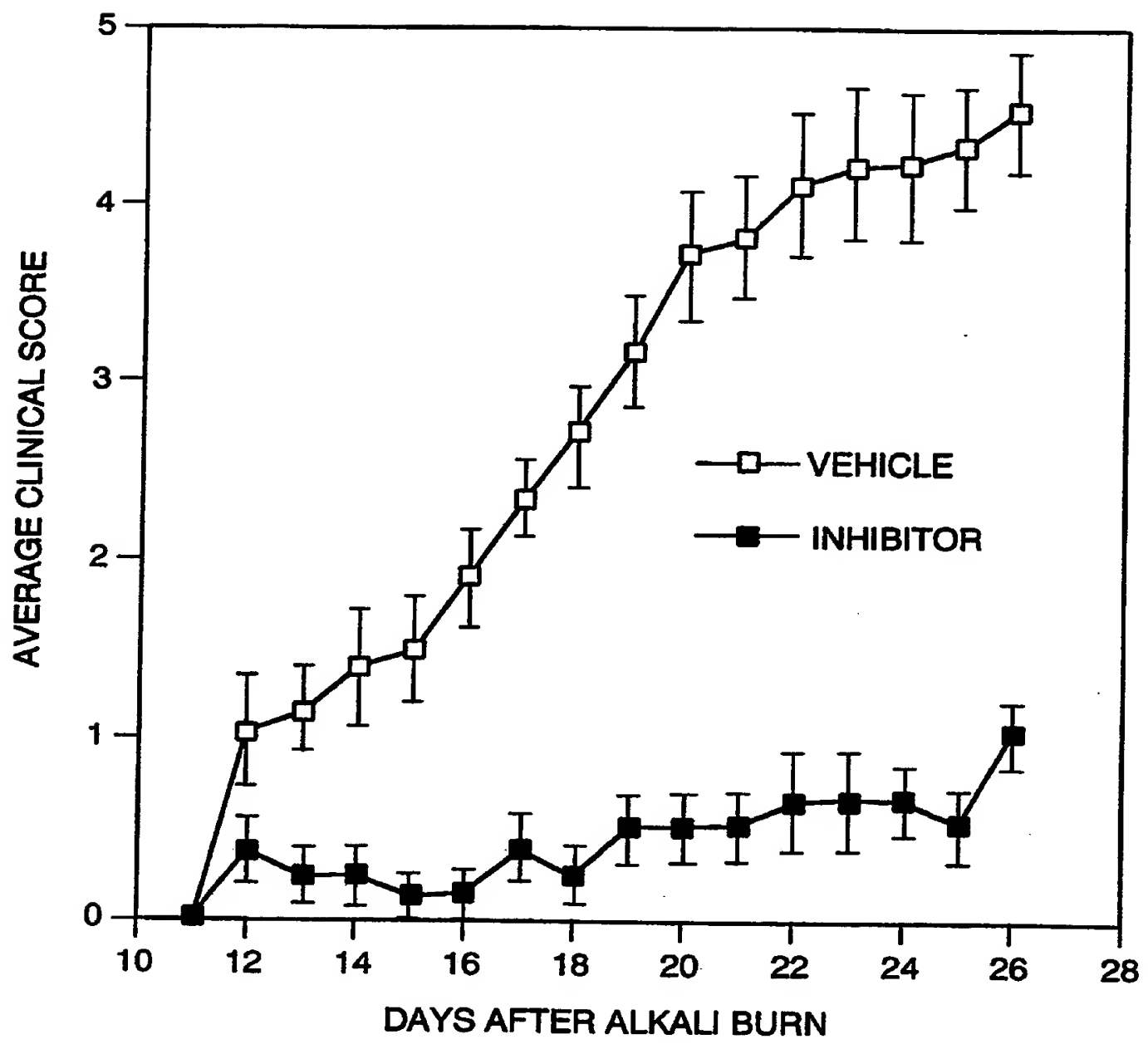
20

wherein each R^6 is independently alkyl (1-4C), under conditions wherein the compound of formula 1 is obtained from the compound of formula 7 or the compound of formula 2 is obtained from the compound of formula 8.

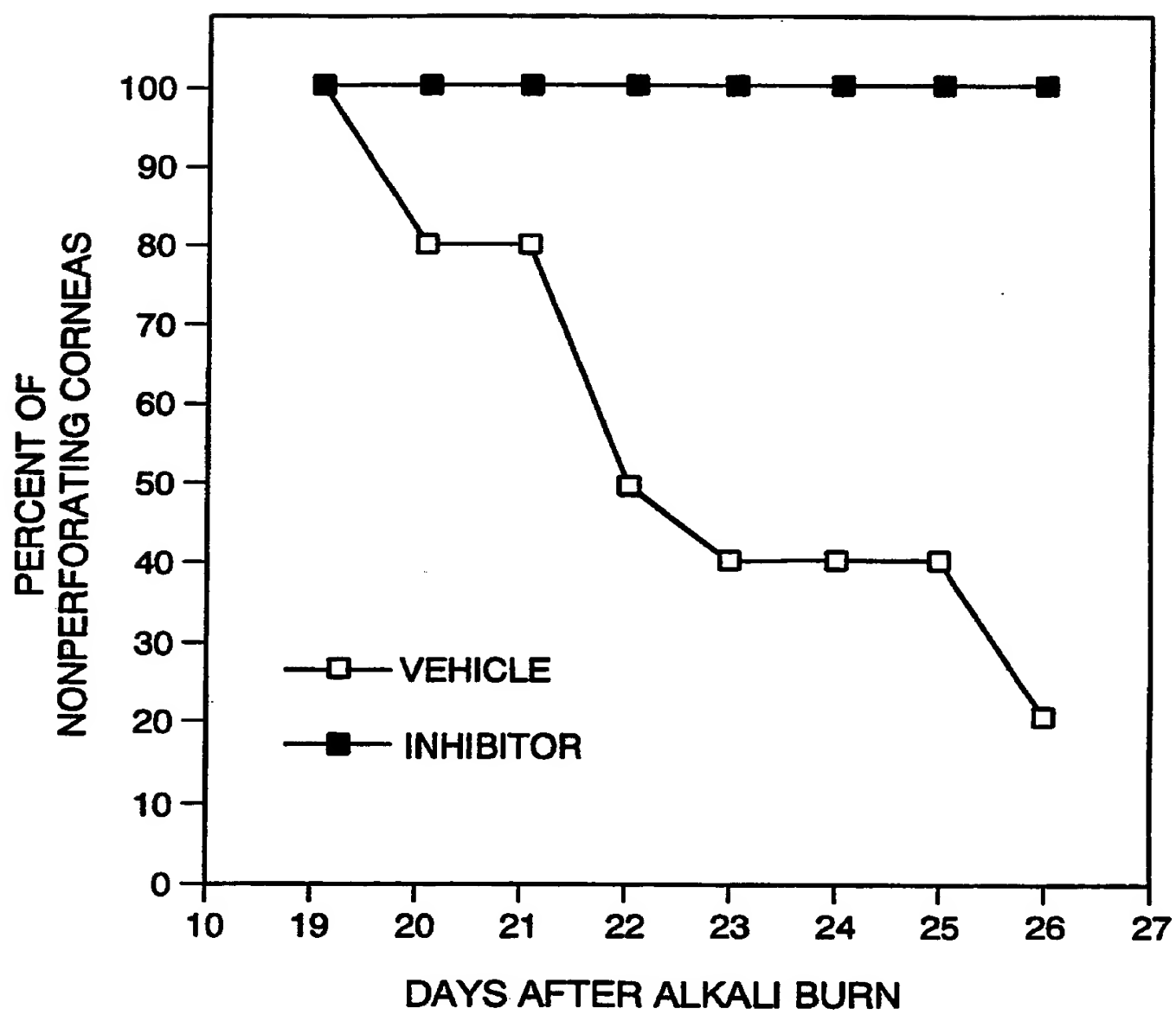
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**FIG. 1**

SUBSTITUTE SHEET

**FIG. 2**

SUBSTITUTE SHEET

INTERNATIONAL SEARCH REPORT

International Application No. PCT/US91/08722

I. CLASSIFICATION OF SUBJECT MATTER (If several classification symbols apply, indicate all) *

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC(5): C07C 259/00 A61K 31/085; C07D 209/20; A61K 31/185; A61K 31/40
 US CL : 548/495; 562/623; 560/312; 514/419; 514/575, 514/5

II. FIELDS SEARCHED

Minimum Documentation Searched ?

Classification System	Classification Symbols
U.S.	548/495; 562/623; 560/312; 514/575 514/419; 514/507

Documentation Searched other than Minimum Documentation to the extent that such documents are included in the Fields Searched *

III. DOCUMENTS CONSIDERED TO BE RELEVANT *		
Category *	Citation of Document, ** with indication, where appropriate, of the relevant passages 12	Relevant to Claim No. 12
Y	US, A, 4,943,587 (CETENKO) 24 JULY 1990 See entire document particularly col. 5, line 51-col. 6,1,35.	4, 6&7
Y	US, A, 4,918,105 (CARTWRIGHT) 17 APRIL 1990 See entire document particularly col. 2, 1,4-col. 3, line 59.	4, 6&7
Y	US, A, 4,681,894 (MURRAY) 21 JULY 1987 See entire document.	4, 6&7

- * Special categories of cited documents: **
- "A" document defining the general state of the art which is not considered to be of particular relevance
 - "E" earlier document but published on or after the international filing date
 - "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
 - "O" document referring to an oral disclosure, use, exhibition or other means
 - "P" document published prior to the international filing date but later than the priority date claimed

- "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- "X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step
- "Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
- "A" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
12 MARCH 1992	14 APR 1992
International Searching Authority	Signature of Authorized Officer
ISA/US	DAVID B. SPRINGER

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

V. ☐ OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE¹

This international search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1. ☐ Claim numbers because they relate to subject matter¹² not required to be searched by this Authority, namely:

2. ☐ Claim numbers because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out¹³, specifically:

3. ☐ Claim numbers because they are dependent claims not drafted in accordance with the second and third sentences of PCT Rule 6.4(a).

VI. ☒ OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING³

This International Searching Authority found multiple inventions in this international application as follows: as set forth in the PCE/ISA/206 (U.S. version) of 2-86, attached.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.
2. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:
3. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers: 4, 6&7 indicated they would be
4. ☐ As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

- ☐ The additional search fees were accompanied by applicant's protest.
☐ No protest accompanied the payment of additional search fees.

CONTINUATION SHEET

INVENTION I - to certain indolyl compounds of generic claim 1,2 and all indolyl compounds in claim 4 with "Trp" (tryptophane group) as well as certain non-hetero containing species of claim 4, I.G., containing Ala (2-naphthal) (alamine) and pharmaceutical compositions (I.6) and methods of use thereof in claim 7.

INVENTIONS II- VI drawn to compounds with certain other heterocyclic groups named in claim 2, but also contained in claim 1, as well as compositions (c16) and methods of use thereof in claim 7.

INVENTION II-having isoquinolul groups

INVENTION III- quinolul groups

INVENTIONS IV- pyrimidinyl groups

INVENTIONS V- comarinyl groups
(and none of I-V having other heterocyclic groups as defined by R⁵ in claim 1)

INVENTIONS VI-having other heterocyclic groups as embraced by cyclic, amine or heterocyclic amine and compositions and methods of use thereof as in claims 6 and 7.

INVENTIONS VII-XII- to certain indolyl and other compounds with groups named by groups.

I-VI wherein "cyclic amines" are present and include at least piperidinyl as named at page 8, line 21 of the specification and compositions and methods of use thereof as in claims 6 and 7.

INVENTIONS XIII-XVIII- to certain indolyl and other compounds with groups named in groups I-VI where heterocyclic amines are present and include at least morpholinyl and compositions (page 8, line 23 of specification) and methods of use thereof (claims 6 and 7).

INVENTIONS XIX-XXXV- to biochemical conjugates of the compound inventions of groups I-XVIII as embraced in claims 1-4 and specifically referred to in claim 4, as embraced by claim 5.

INVENTIONS XXXVII-LIV- to methods of inhibiting metallo protease invitro or in cell cultures (in vivo) using compounds embraced in claims 1-4, and as set forth in claim 8 embracing the compounds in Inv. I-XVIII.

CONTINUATION SHEET # 2

INVENTIONS LV-LXXII- to various immuno reactive anti-body preparations of the compounds of claims 1-4 as set forth in claim 9 embracing the compounds of I-LXXII as set forth above. Inventions LXXIII-LC to method(s) of assaying antibodies by complexes thereof with the compounds of claims 1-4, and as set forth in claim 10.

INVENTIONS LCI-LCVI- to six materially different methods of preparing the compounds of claim 1 as enumerated by claims 11-13 respectively. The above enumerated ninety-six (96) inventions lack unity of invention since they do not form a single inventive concept in that a reference for any one of the compound compositions and method of use inventions of the groups would not render obvious any other, and searches for the various inventions are not coextensive.

The conjugate inventions of XIX-XXXVI are obviously distinct from invitro matrix and cell culture matrix metallo protease inhibition as are the immuno-reactive antibody and assaying methods inventions.

The materially different method of Groups LXXIII-LC of claims 11-16, for preparing variously the compounds of claim 1 are evidence each of these six methods is distinct from each other and each from any of the compounds of Inventions I-XXII prepared thereby. For the above reasons, lack of unity of invention has been shown in accordance with 37 CFR 1.481(d) so that the above identified inventions are patentably distinct, have acquired a separate status in the art, and require different fields of search.

It is noted that the additional searches for the ninety-five additional inventions requires payment of \$15,200.00 (95 x \$160.00) (somewhat higher than the \$150.00/invention previously). This draft copy was necessitated by the need to provide in writing as soon as possible the unity of invention invitation. Ms. Murashige indicated in a telecon 3/4/92 that it would be decided shortly after a fax copy was received and that they would promptly notify the examiner as to their decision.

On Tuesday March 10, 1992 in a telephone conversation with Atty., Ms. Murashige additional fees were not authorized but it was requested all species of claim 4 and 6&7 corresponding to compositions and methods thereof be searched to which the examiner agreed necessitating the modification of this invitations as herein to include all species into invention I. Thus naphthyl substituted hydroxamic acids & esters 562/623 and 560/312 as well as corresponding 514/507 and 514/575 were searched.